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## STUDIES ON SOIL PHYSICS.

PART II.—THE PERMEABILITY OF AN IDEAL SOIL TO  
AIR AND WATER.

BY HEBER GREEN AND G. A. AMPT.

*Introduction.*

§ 1. In Part I. (vol. iv. pp. 1—24) it was shown that the intrinsic permeability of a soil when measured with water was less than when measured with air as the experimental fluid, and that the ratio of the two values varied with the amount of colloidal matter present. It was desirable to determine accurately whether the values would be identical for soils composed of pure sand or other uniform and non-colloidal particles.

It should also be possible to calculate the permeability of a soil from a knowledge of the sizes of its component particles and of the specific pore-space; and this problem is one that has engaged the attention of several authors both from the experimental and mathematical standpoints.

*Previous Investigations.*

§ 2. Allen Hazen (*Ann. Rep. State Board of Health, Mass., U.S.A., 1892*), in an investigation of filter-bed sands and gravels, used an experimental filter filled with various grades of sands, and deduced the formula

$$v = cd^3 \frac{h}{l} (0.70 + 0.03\theta),$$

where  $c$  is a constant, approximately 1000;  $d$  is the effective diameter of sand grain;  $h$  is the head of water pressure;  $l$  is the length of sand column;  $\theta$  is the temperature; and  $v$  is the velocity of water-flow in metres daily.

§ 3. King and Slichter (*Nineteenth Annual Report*, 1899, Part 2, of the U.S. Geol. Survey), in a very complete and masterly research, have not only experimentally measured the permeability of sands, soils and rocks to both air and water, but the latter has developed a mathematical solution of the problem.

Slichter's mathematical argument may be summarized as follows:—

If we consider a soil composed of spherical particles, then the lines joining any eight contiguous spheres will be found to outline a parallelepiped varying in form from a cube to a rhombohedron, according as the particles are packed in the loosest or closest manner. This unit element of volume with length of side  $d$  will contain the equivalent of a single sphere of diameter  $d$ .

"If the grains of soil are arranged in the most compact manner possible, each grain will touch surrounding grains at twelve points, and the elements of volume will be a rhombohedron having face angles equal to  $60^\circ$  and  $120^\circ$ ." "If the grains are not arranged in the most compact manner, the rhombohedron will have its face angles greater than  $60^\circ$  and each sphere will touch other spheres in but six points and *nearly* touch in six other points." "The most open arrangement of the soil grains, which is possible with the grains in contact, is had when the rhombohedron is a cube."

For a rhombohedron whose side is of unit length the volume is given by  $(1 - \cos \theta)(\sqrt{1 + 2 \cos \theta})$ , where  $\theta$  is the face angle; and consequently the pore-space  $S$ , not occupied by the enclosed sphere, is given by

$$S = 1 - \frac{\frac{\pi}{6}}{(1 - \cos \theta)\sqrt{1 + 2 \cos \theta}} \dots\dots\dots(1).$$

The values of  $S$  for different values of  $\theta$  are thus readily calculable and are given in Table I.

Slichter finds, that if  $l$  be the length of the soil column under consideration and  $d$  the diameter of each particle, then the length of the pore-space capillary may be taken as equal to  $\frac{l(1 - \cos \theta)}{\sin \theta \sqrt{1 + 2 \cos \theta}}$ , and the area of its minimum cross-section as equal to

$$\frac{\sin \theta - \frac{\pi}{4}}{2} \cdot d^2.$$

A mathematical and experimental investigation showed that the error involved in assuming the pore to be *circular and equal in area*

to the minimum cross-section of the actually triangular pore is almost balanced (to within one per cent.) by the assumption that the pore is straight instead of curved.

Hence, using the same nomenclature as in Part I. (*loc. cit.* § 6), we may write Poiseuille's equation

$$\frac{v}{t} = \frac{\pi}{8\eta} \cdot \frac{ghs}{l} \cdot \Sigma r^4 \dots\dots\dots(2)$$

in the form

$$\begin{aligned} \frac{v}{t} &= \frac{\pi}{8\eta} \cdot \frac{ghs \sin \theta \sqrt{1+2 \cos \theta}}{l(1+\cos \theta)} \cdot \frac{\left(\sin \theta - \frac{\pi}{4}\right)^2}{4\pi^2} \cdot d^4 \\ &= \frac{ghsd^4}{32\eta l} \cdot \frac{\sin \theta \sqrt{1+2 \cos \theta}}{1+\cos \theta} \cdot \left(\sin \theta - \frac{\pi}{4}\right)^2 \dots\dots\dots(3), \end{aligned}$$

for each of the two pores penetrating a unit element, *i.e.* the rate of flow per area  $\frac{\sin \theta}{2} \cdot d^2$  which is half the area of cross-section occupied by each rhombohedron.

Then if the cylinder containing the column of soil be of area  $A$ ,

$$\begin{aligned} \frac{v}{t} \text{ (for the whole area)} &= \frac{ghsAd^2}{16\pi\eta l} \cdot \frac{\sqrt{1+2 \cos \theta}}{1+\cos \theta} \cdot \left(\sin \theta - \frac{\pi}{4}\right)^2 \\ &= \frac{ghsAd^2}{16\pi\eta l} \cdot \frac{(1-\cos \theta)(\sqrt{1+2 \cos \theta})\left(\sin \theta - \frac{\pi}{4}\right)^2}{\sin^2 \theta} \dots\dots\dots(4). \end{aligned}$$

But from equation (1)

$$1-S = \frac{\frac{\pi}{6}}{(1-\cos \theta)(\sqrt{1+2 \cos \theta})};$$

therefore

$$\begin{aligned} \frac{v}{t} &= \frac{ghsAd^2}{96\eta l} \cdot \frac{\left(\sin \theta - \frac{\pi}{4}\right)^2}{(1-S) \sin^2 \theta} \\ &= \frac{ghsAd^2}{96\eta l} \cdot \frac{\left(1 - \frac{\pi}{4} \operatorname{cosec} \theta\right)^2}{1-S} \dots\dots\dots(5). \end{aligned}$$

Put  $B = 1 - \frac{\pi}{4} \operatorname{cosec} \theta$ , and take  $g = 980$  and  $s = 1$ , then

$$\eta \cdot \frac{v}{th} \cdot \frac{l}{A} = 10 \cdot 2d^2 \cdot \frac{B^2}{1-S} \dots\dots\dots(6),$$



and putting 
$$k = \frac{1 - S}{B^2},$$

we get 
$$\eta P = 10.2 \frac{d^2}{k} \dots \dots \dots (7).$$

The values of  $S$  and the mathematically derived constants  $B$  and  $k$  were calculated by Slichter for various values of  $\theta$  and are given in Table I. It will be seen that  $k$  varies from 11.37 for the loosest packing to 85.43 for the most compact arrangement possible with perfect spheres.

TABLE I.

*Values of the "permeability constant" ( $k$ ), as calculated by Slichter, for all possible variations of pore-space ( $S$ ) when dealing with a soil composed of spherical particles.*

$S$ Pore-space	$\theta$ Angle of packing	$B$ $= 1 - \frac{\pi}{4} \operatorname{cosec} \theta$	$k$ $= \frac{1 - S}{B^2}$
.2595	60° 00'	.0934	85.43
.30	62° 36'	.1155	52.45
.31	63° 18'	.1210	47.1
.32	64° 3'	.1266	42.45
.33	64° 49'	.1322	38.15
.34	65° 37'	.1378	34.75
.35	66° 27'	.1434	31.6
.36	67° 21'	.1491	28.8
.37	68° 18'	.1549	26.26
.38	69° 17'	.1605	24.08
.39	70° 20'	.1661	22.1
.40	71° 28'	.1719	20.3
.41	72° 43'	.1775	18.75
.42	74° 3'	.1832	17.3
.43	75° 32'	.1890	15.95
.44	77° 10'	.1946	14.75
.45	79° 6'	.2003	13.70
.46	81° 25'	.2057	12.75
.47	84° 59'	.2117	11.83
.4764	90° 00'	.2116	11.37

#### § 4. The desirability of testing the formula

$$\eta P = 10.2 \frac{d^2}{k}$$

experimentally for both air and water is self-evident and King has carried out, in conjunction with Slichter, an elaborate series of measurements with sands of various sized grains.

A summary of the results obtained in one series [*Fifteenth Annual Report*, Agr. Expt. Stn. Univ. Wisconsin, p. 127] is given in Table II.

TABLE II.

*Summary of experiments, by F. H. King, on the Permeability of Sands to Air and Water.*

Measured diameter of sand grains	Permeability to water calculated from			Average percentage error
	known diameter of grains	observed flow of air	observed flow of water	
	$P_w = c \frac{d^2}{\eta_w k}$	$P_w = P_a \frac{\eta_w}{\eta_a}$	$P_w = \frac{v}{t}$	
2.755	2680	2277	2296	- 17.5
1.993	1372	1132	1080	- 24
1.588	909.1	757	756	- 20
1.345	638.6	522	542	- 20
1.157	499.6	453.2	504.6	- 4.5
1.106	326.6	297.5	329.2	- 4
.892	191.0	193.0	210.0	+ 3.5
.665	106.2	122.0	138.6	+ 22.5
.582	75.7	80.6	94.8	+ 16
.489	59.8	66.8	72.3	+ 16

They confirm the formula to within a little more than experimental error, but it must be borne in mind that King's apparatus allowed a very considerable margin for this factor. King himself admits these discrepancies and goes on to say,—“Search has been made thus far in vain for some medium consisting of spherical grains of perfectly uniform diameter, with which to secure a rigid test of the method, but, as yet, none has been found.”

Shot are unsuitable, partly on account of their weight, but also because the finest dust shot the present authors can obtain have a diameter of about 1 mm. and much finer sizes are required.

### *Preparation of Material.*

§ 5. *A suitable material has however been found* in the “glistening dew” of the picture post-card artist. This is composed of almost perfectly spherical grains or beads of glass ranging in diameter from 0.25 mm. upwards. Enquiries have been made for “beads” of still smaller sizes, but such are apparently not manufactured; if obtainable

they would be extremely valuable for an extension of this and similar researches on soil physics and the phenomena of adsorption.

Two varieties were used; the smaller of which (0.5 mm. or less) were composed of colourless glass, often surface stained with aniline dyes, whilst the larger (up to 1 mm.) were coloured with various metallic oxides.

Sifting was soon found to be a tedious method of grading them, for the sieves became almost immediately choked and were with difficulty freed from beads which were but little larger than the perforations.

§ 6. Finally an elutriation method was devised, based on the ordinary processes used for the mechanical analysis of soils.

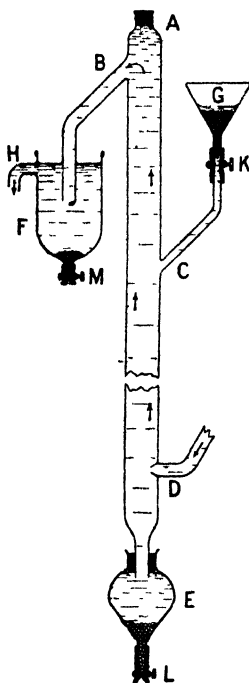


FIG. 1. Elutriator for grading beads and sands.

The apparatus<sup>1</sup> consisted of a vertical tube *ABCDE* about six feet long and one inch in diameter, provided with an inlet tube at *D* with a constricted jet for the water supplied from a constant level reservoir.

The water passed upwards and out at *B* to the cylinder *F*, finally escaping to the waste by overflowing at *H*. After they have been

<sup>1</sup> We are indebted to Mr Radcliff of the Bairnsdale School of Mines for suggesting this shape of elutriating tube.

cleaned by successive treatment with caustic soda and aqua regia, the beads to be graded are poured into the funnel *G* and flow in a stream, regulated by the clip *K*, through *C* into the main elutriation tube where they meet the upflowing current of water.

Those beads having a diameter greater than the "critical diameter" for the water current will sink past *D* into *E* and can be drawn off through *L* as required: the lighter beads will rise and be carried over into *F* where they will settle and may be removed by opening the clip *M*.

Many precautions were found necessary, as Hilgard has pointed out for his soil elutriator, and even under the best conditions it is impossible to obtain a perfect separation. When many beads are present in the tube *BC* it will no longer have the same efficient area as when empty of beads, and so the velocity of upward flow of the water becomes materially increased and beads having more than the critical diameter will be carried over into *F*. Another cause of error is the variation in stream velocity with the distance from the walls of the tube and often beads may be observed travelling upwards for several inches in mid-stream, and then, after approaching the wall, tumbling downwards again.

Occasionally beads have hovered about in the tube for two or three days, but even these on microscopical examination exhibit a considerable variation in their diameters.

§ 7. The separations obtained were the results of many repetitions of this elutriation process, care being taken to put each sample through slowly. The original mixture was thus classified into thirteen grades, of which five were selected for the final experimentation.

§ 8. The average diameter of the beads in each grade was determined by counting at least two thousand beads and taking weighings at regular intervals. As each bead was separately picked out with a small pair of forceps and transferred to a weighing bottle the process became very tedious, especially in the case of the smaller sizes. In all, some 40,000 beads were counted out in this way—one by one.

The density of each grade was determined by displacement of water in a specific gravity bottle—it was found to vary considerably with the size. The two larger sizes being coloured with metallic oxides showed a corresponding increase of density.

The density of these beads was found to be 3.117 and their average diameter was therefore 0.9374 mm.

The discrepancy between the weights of the separate lots of beads

was greater than would have been expected either from the method employed in sorting them or from their appearance under the microscope (see Pl. I, figs. 8 and 9). The discrepancy was less in the case of the smaller beads but greater for the sand grains used in the later part of this work. The large numbers counted and weighed must however have eliminated any perceptible error due to this cause.

TABLE III.

*Weight of large coloured glass beads.*

Weight of first thousand=1.3319				Weight of sixth thousand=1.3861			
„	second	„	= 1.3946	„	seventh	„	= 1.3149
„	third	„	= 1.3428	„	eighth	„	= 1.2835
„	fourth	„	= 1.3467	„	ninth	„	= 1.4070
„	fifth	„	= 1.3027				
Average weight per thousand=1.3456							

*Standardization of Apparatus for measuring Permeability.*

§ 9. As the tubes filled with beads had a very much larger permeability than the soils previously dealt with, the rates of flow for both air and water no longer conformed to Poiseuille's simple capillary tube law<sup>1</sup> and were sufficiently high to make the correction for loss of kinetic energy of the moving fluid an appreciable factor.

In order to determine the value of this and other corrections, and to test the accuracy of the method, the special apparatus employed was first used to measure the permeability of a straight capillary tube whose constants could be exactly obtained from its dimensions.

This capillary tube was carefully calibrated with a thread of clean mercury: the variations in diameter amounted to less than one per cent.

Length of capillary = 54.90 cm.

Average area of cross-section = 0.00745 sq. cms.

Then using the same nomenclature as in Part I. of this paper [*vide* this *Journal*, vol. iv. pt I. pp. 3—6], from Poiseuille's equation

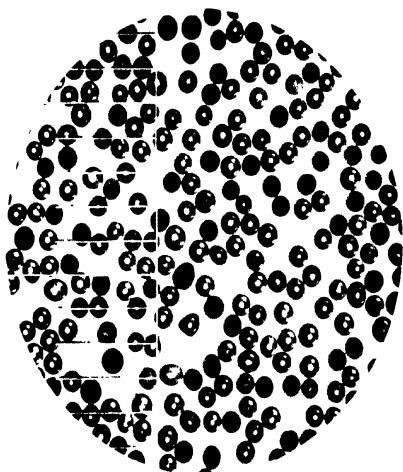
$$\eta = \frac{r^4 \pi g}{8} \cdot \frac{hst}{lv},$$

and taking

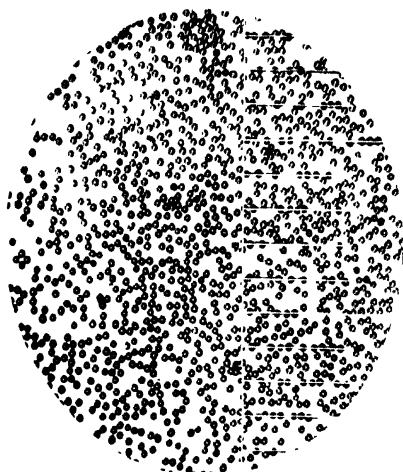
$$P = \frac{v}{t} \cdot \frac{l}{hs},$$

$$\eta P = \frac{\pi g}{8} \cdot r^4.$$

<sup>1</sup> See Vol. iv. p. 3, § 6.

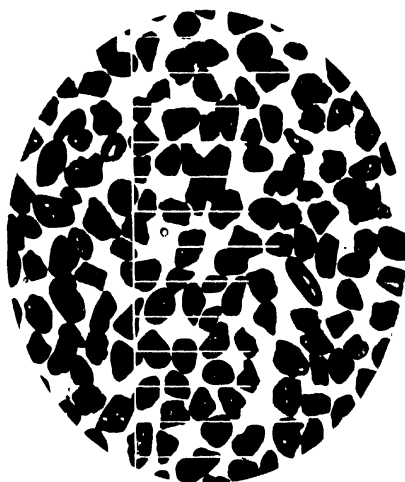


Grade B : diameter = 0.709 mm

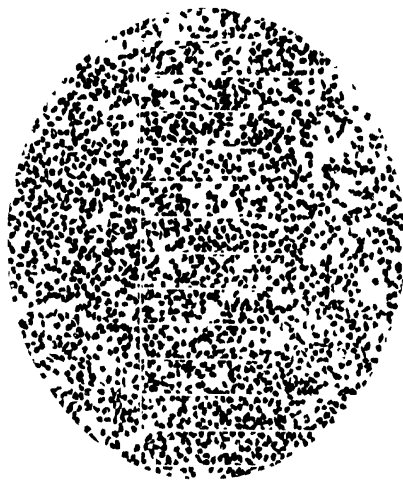


Grade E : diameter = 0.250 mm.

Fig. 8.



Grade a : diameter = 0.825 mm.



Grade c : diameter = 0.186 mm

Fig. 9.



Therefore, for the tube in question,

$$\eta P = 0.002153.$$

§ 10. *Permeability to air.* The corrections applied were:—

(i) *For loss of kinetic energy.* The well-known Couette-Finkener formula is

$$\eta = \frac{\pi}{8} \cdot \frac{ghst}{vl} \cdot r^4 - \frac{\rho}{8\pi} \cdot \frac{v}{lt},$$

where  $\rho$  is the density of the fluid (air); then as

$$\begin{aligned} P_{\text{obs.}} &= \frac{vl}{th}, \\ P_{\text{corr.}} &= P_{\text{obs.}} \left( 1 + \frac{\rho}{8\pi} \cdot \frac{v}{\eta lt} \right) \\ &= P_{\text{obs.}} \left\{ 1 + \frac{\rho P_{\text{obs.}}^2}{\pi^2 l^2 gr^4} \cdot h \right\}, \end{aligned}$$

which for the capillary tube in question simplifies to

$$P_{\text{corr.}} = P_{\text{obs.}} (1 + 0.00107h).$$

(ii) *For compressibility of the air.* Meyer, Breitenbach and others have shown that Poiseuille's law when applied to gases should take the form

$$\eta = \frac{\pi}{8} \cdot \frac{r^4 t}{vl} \cdot \frac{p_1^2 - p_2^2}{2p_0},$$

where  $p_0$  is the pressure under which the air is measured;

$$\begin{array}{llll} p_1 & \text{,,} & \text{,,} & \text{of air at the high pressure end of the capillary;} \\ p_2 & \text{,,} & \text{,,} & \text{low ,, ,, ,, ,,} \end{array}$$

As pressures were measured in centimetres of water in the gauge, this correction reduced to

$$v_{\text{corr.}} = v_{\text{obs.}} \left( 1 + \frac{2h_0 - h}{2B + h} \right)$$

for experiments in which a "head" of pressure was employed, and to

$$v_{\text{corr.}} = v_{\text{obs.}} \left( 1 + \frac{h - 2h_f}{2B - h} \right)$$

for experiments in which a "tail" of pressure was employed, i.e. where the pressure in the bulb  $C$  was less than atmospheric.

$B$  = barometric pressure expressed in cm. of water;

$h$  = mean working pressure;

$h_0$  = initial pressure in measuring bulb;

$h_f$  = final pressure in measuring bulb.



(iii) Corrections were also applied for the “*dead space*” in the connecting tubes between the measuring bulb and the capillary tube, and for the slight alteration in volume due to movement of the water in the pressure gauge.

All these corrections were summarized for our apparatus in the following equation, which, though complicated in appearance, was found to be simple in application :

$$P_{\text{corr}} = P_{\text{obs.}} (1 + 0.00107h) \left[ 1 + \frac{2h_0 - h}{2B + h} \text{ or } 1 + \frac{h - 2h_f}{2B - h} \right] \left\{ 1 + \frac{1}{v} \left( \frac{h_0 - h_f}{20} + \frac{h_0 - h_f}{14} \right) \right\}.$$

§ 11. The apparatus, shown in Fig. 2, consisted of a measuring bulb *C*, of known capacity between the marks *B* and *D*, connected on

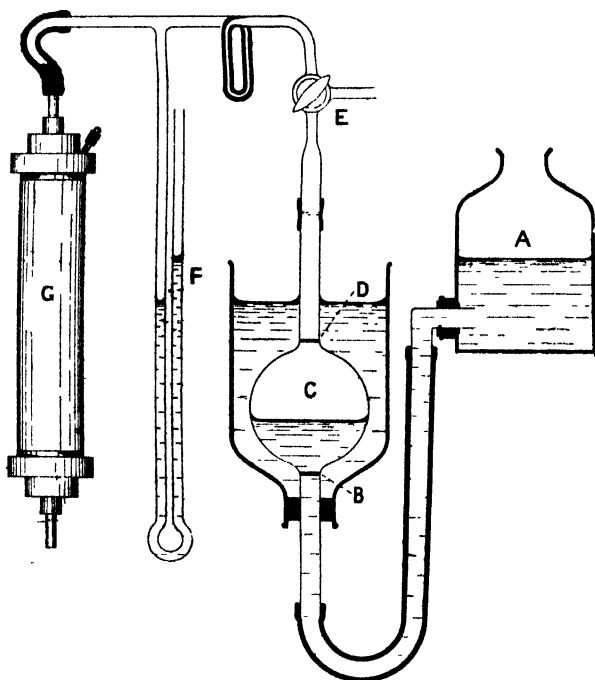


FIG. 2. Apparatus used for the measurement of the permeability to air of a glass tube filled with beads.

the one side to an adjustable water reservoir *A*, and on the other to the tube *G* containing the glass beads (or the capillary tube) whose permeability to air was to be measured.

To carry out an experiment the reservoir *A* was adjusted so as to give the required pressure and, by means of the three-way tap *E*, air was either forced or aspirated through until the water level was several centimetres either below *B* or above *D* according as the measurements were to be made under "head" or "tail" of pressure. The tap *E* was then turned so as to make communication between *C* and *G*, and the water level rapidly rose as the air in *C* was being forced through *G*. At the moment of passing *B* the stopwatch was started and the first reading of the pressure at *F* obtained by means of a reading telescope; further readings were made at regular time intervals and the watch stopped as the water level passed *D*.

The mean pressure *h* was obtained by averaging these readings, half weight only being given to the initial and final pressures (*h*<sub>0</sub> and *h*<sub>*f*</sub>).

Whilst the temperature coefficient for the viscosity of air is only small, yet it is necessary to waterjacket the bulb *C* to prevent *changes* of temperature, and therefore of effective volume, *during* the progress of an experiment. For a similar reason *C* and *G* must be maintained at the *same* temperature; consequently, when the capillary tube was being experimented on, it was enclosed in a Liebig's condenser through which the water that had circulated round *C* was passed.

TABLE IV.

Time readings	<i>h</i> (cm.)	
	(a) using "head" of pressure	(b) using "tail" of pressure
Initial	9.75	10.7
15"	7.4	8.1
30"	6.7	7.45
45"	6.1	6.8
1' 0"	5.6	6.2
1' 15"	5.15	5.7
1' 30"	4.75	5.25
1' 45"	4.35	4.75
2' 0"	3.95	4.3
2' 15"	3.55	3.85
2' 30"	3.25	3.35
2' 45"	2.85	2.8
3' 0"	2.4	—
3' 15"	2.0	—
Final	1.05	1.9
Mean value of <i>h</i>	4.53	5.41

Temp. = 9.7° C.

(a) Using "head" of pressure ;  
*t* = 3.425 mins.  
*h* = 4.53 cm.

(b) Using "tail" of pressure ;  
*t* = 2.855 mins.  
*h* = 5.41 cm.

A typical set of readings are given in Table IV for a pair of experiments in which a "head" and "tail" of pressure respectively were used.

§ 12. A number of experiments were made with this capillary tube under widely different conditions of pressure in order to decide whether the formulae given by Meyer and others would hold good for our apparatus and eliminate the various errors due to loss of kinetic energy etc.

TABLE V.

*Measurements of the Permeability (to air) of a glass capillary tube.*

$t$ (secs.)	$h$ (cms.)	$P_{\text{obs.}}$	$h_0$	$h_f$	$P_{\text{corr.}}$	Temp.	$\eta \times 10^6$	$\eta P_{\text{corr.}}$
(i) Using "head" of pressure								
54.8	+ 17.84	11.64	23.4	14.5	12.10	8.8°	177.2	.002144
67.0	14.47	11.74	19.9	11.0	12.13	8.8°	177.2	2148
83.1	11.57	11.85	17.0	8.15	12.19	10.0°	177.8	2168
100.6	9.44	11.96	14.8	5.95	12.26	9.0°	177.2	2172
125.7	7.51	12.00	12.95	4.05	12.27	9.0°	177.2	2174
171.9	5.47	12.09	10.9	2.0	12.31	9.4°	177.4	2181
215.5	4.35	12.22	9.85	1.05	12.36	9.4°	177.4	2192
250.4	3.67	12.38	9.25	0.5	12.38	9.8°	177.7	2236
(ii) Using "tail" of pressure								
50.3	- 19.28	11.83	24.8	16.0	12.06	10.5°	178.0	.002146
53.5	17.89	11.87	23.35	14.5	12.10	9.7°	177.7	2152
58.9	16.24	11.88	21.6	12.75	12.12	9.9°	177.7	2152
67.1	14.18	11.93	19.5	10.7	12.13	10.1°	177.8	2154
69.3	13.61	12.03	18.95	10.1	12.23	9.6°	177.6	2176
80.3	11.83	11.95	17.0	8.2	12.13	10.1°	177.8	2154
96.3	9.86	11.97	15.0	6.1	12.15	9.6°	177.6	2156
117.6	8.015	12.06	13.05	4.3	12.22	9.6°	177.6	2170
140.0	6.71	12.10	11.8	3.05	12.26	9.7°	177.7	2178
171.3	5.41	12.28	10.7	1.9	12.43	9.7°	177.7	2208
205.4	4.53	12.23	9.75	1.05	12.36	9.7°	177.7	2196

§ 13. Both of these series of experiments (in Table V) exhibit a slight regular progression, and seem to indicate that the various corrections leave some residual effect unbalanced. It was found difficult to eliminate the error due to differing surface tension forces acting on the water in the two limbs of the manometer; but the zero error, sometimes 0.1 or 0.2 mm., was always read and allowed for.

The true value of  $\eta P$  should apparently be obtained by working at an infinitely small pressure or by extrapolation of the values obtained

at higher pressures, but owing to the shape of the measuring bulb the lowest mean pressure that could be worked with, in the manner described above, was about 4 cms. The device was therefore adopted of inserting a "resistance in the circuit," between  $E$  and  $G$ , in the shape of a suitable piece of capillary tubing. This allowed of mean pressures of less than 1 cm. being utilized without loss of accuracy although initial and final readings of the pressure in the bulb ( $h_0$  and  $h_f$ ) as well as at the manometer ( $h_1$  and  $h_2$ ) had to be taken in order to apply the usual corrections indicated in § 10.

TABLE VI.

*Measurements of the Permeability (to air) of a glass capillary tube, using a "resistance" capillary in the circuit.*

$t$ (secs.)	$h$ (cms.)	$P_{\text{obs}}$	$h_0$	$h_f$	$h_1$	$h_2$	Temp.	$\eta P_{\text{corr}}$
216.9	-4.299	12.23	23.5	14.7	5.37	3.41	10.0°	.002166
216.7	-4.276	12.28	23.5	14.7	5.37	3.41	8.5°	2164
240.9	+3.960	11.90	22.1	13.3	5.16	3.09	10.0°	2174
240.9	+3.960	11.92	22.1	13.3	5.16	3.09	9.0°	2170
603.7	+1.579	12.15	12.0	13.1	2.82	0.73	9.8°	2194
607.5	-1.512	12.35	11.5	2.6	2.69	0.64	9.4°	2198
990.7	-0.940	12.20	9.3	0.4	2.20	0.13	10.2°	2195
1053.5	-0.887	12.17	9.5	0.6	2.18	0.10	10.2°	2192

§ 14. All the values of  $\eta P_{\text{corr}}$  obtained are shown in Fig. 3 and it will be seen that the "gang" is independent of experimental error and that the results for both "head" and "tail" pressures, with and without

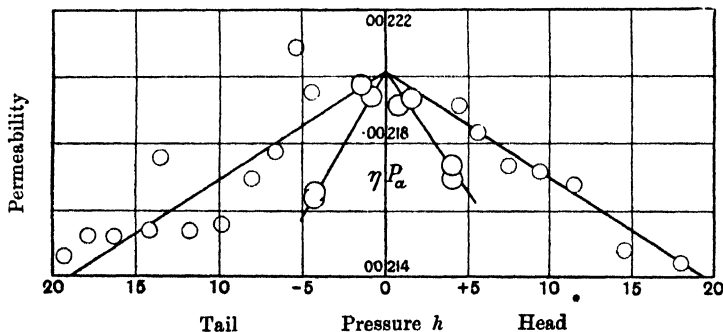


FIG. 3. Permeability of capillary tube to air under varying pressures.

the capillary in series, all extrapolate almost exactly to  $\eta P = 0.002202$ . This is 2.2 per cent. greater than 0.002153 the value calculated from

the measured dimensions of the capillary and taking  $\eta_0 = 0.0001730$  [Meyer and Breitenbach]; but it must be borne in mind that the absolute value for the viscosity of air obtained by different observers varies by at least this amount.

Consequently we may fairly claim for this apparatus an accuracy quite sufficient for our needs.

§ 15. *Permeability to water.* The water was supplied from a large reservoir and after passing through the capillary was, in the preliminary experiments, allowed to drip from the curved exit tube into the receiving vessel—the head of pressure being taken as the difference in level between the orifice of this exit tube and the water in the reservoir.

The capillarity effects at the outlet are considerable however, and were overcome by reading the pressure from two side tubes connected to each end of the tube whose permeability is being measured. The nozzle of the outlet tube had also to be immersed in liquid and placed in contact with the wall of the burette that was used as a receiving vessel, otherwise a fluctuation of pressure was observed as each drop detached itself from the orifice.

The results obtained in a series of experiments at different pressures are given in Table VII. The decrease of  $\eta P_w$  at low pressures is to be accounted for by the loss due to evaporation of the effluent water, and the percentage error due to this cause will evidently be proportional to the reciprocal of the rate of flow or, more conveniently, driving pressure.

TABLE VII.

*Measurement of the Permeability (to water) of a glass capillary tube.*

$h$ (cms.)	$\eta_w P_w$	$\frac{1}{h}$
19.39	0.002158	.052
11.21	2148	.089
10.41	2147	.096
10.20	2143	.098
4.119	2107	.243
3.025	2087	.331
2.048	2028	.489
1.980	2030	.506
1.098	1907	.911
0.56	1740	1.787

The results obtained for  $\eta_w P_w$  are therefore plotted (in Fig. 4) against  $1/h$  and the graph is, as was expected, a straight line extrapolating to  $\eta P = 0.002173$ .

The kinetic energy correction, even for the highest rates of flow, is almost insignificant and only raises this extrapolated value of  $\eta P$  to 0.002183.

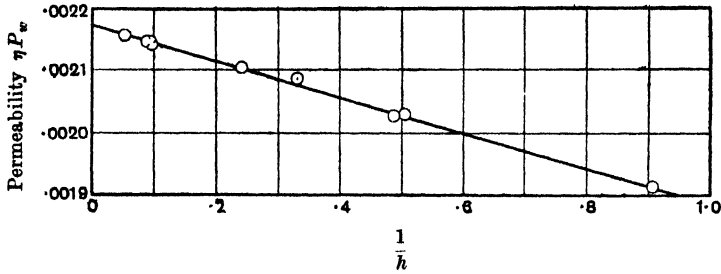


FIG. 4. Permeability of capillary tube to water under varying pressures.

§ 16. All the results obtained with this capillary tube may be summarized thus:

$$\begin{aligned}\eta P & \text{ (calculated from the measurements of the tube)} = 0.002153, \\ \eta_a P_a & \text{ (from observed permeability to air)} = 0.002202, \\ \eta_w P_w & \text{ (from observed permeability to water)} = 0.002183.\end{aligned}$$

The accuracy shown here is abundantly sufficient for the purpose in view.

#### *Construction and Testing of Apparatus for holding Beads.*

§ 17. Many measurements were made of the permeability of each of the various grades of beads when contained in narrow tubes, such as had been previously used for soils, but it was found that the error in the measurement of the pore-space in these narrow tubes was too great for the results to be of value in our present enquiry.

§ 18. A special containing vessel, shown in Figs. 2 and 5, was therefore designed. It consisted of a glass tube with brass cone-shaped end pieces which were carefully ground to fit the glass tube and were capable of exact measurement before being cemented in place. The inner surfaces of these brass ends were turned into hollow cones whose apices were connected with the exterior by means of short pieces of brass tubing into which corks and glass tubes were inserted.

The beads were retained in place by a disc of wire gauze (shown in the figure by a line of dots) and were fed in through a small hole drilled diagonally through the brass ends. This arrangement allowed

of the accurate determination of volume and other dimensions and proved thoroughly satisfactory.

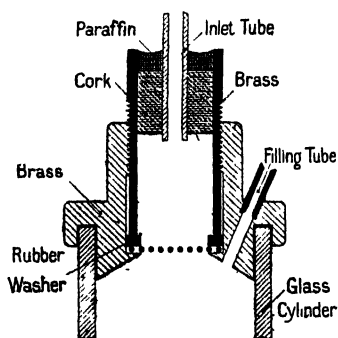


FIG. 5.

A knowledge of the volume is necessary to determine the pore-space  $S$ , and  $\frac{l}{A}$  is also required since it enters into the formula

$$P = \frac{v}{th} \cdot \frac{l}{A}.$$

The value of  $l/A$  was separately determined for the body of the tube and the conical ends.

For a truncated cone

$$\begin{aligned} \frac{l}{A} &= \frac{1}{\pi \tan \alpha} \left( \frac{1}{r} - \frac{1}{R} \right) \\ &= \frac{h}{\pi r R}, \end{aligned}$$

where  $\alpha$  is the angle of the cone,  $r$  and  $R$  the radii of its top and base respectively and  $h$  its length.

§ 19. The measurements of the cones and tubes, made with specially accurate calipers, were as follows for the two vessels that were employed.

Vessel I was only used in the earlier experiments as the glass cylinder was repeatedly breaking. Nearly all the recorded experiments were made with Vessel II which was carefully made to fit a glass tube of better quality and very uniform bore.

§ 20. Bead sample  $D$  (diameter = 0.319 mm.; density = 2.750) was first experimented with. Vessel I was loosely filled with about 200 grams of the beads and its permeability to air measured by means of the apparatus described in § 11; then, by gentle packing, an extra one or two grams of beads were introduced and the permeability again measured. This

process was repeated until, even on continued "dumping" and rotating, the tube would hold no more beads.

TABLE VIII.

*Dimensions of Vessels in which Permeabilities of Beads were measured.*

	Vessel I		Vessel II	
Area of cross-section of glass cylinder...	7.09 cm. <sup>2</sup>		3.567 cm. <sup>2</sup>	
Length of glass cylinder.....	12.13 cm.		23.60 cm.	
Length of cones = $h$ .....	0.394	0.362	0.328	0.342
Radius of base of cones = $R$ .....	1.473	1.470	1.052	1.048
Radius of apex of cones = $r$ .....	0.720	0.720	0.500	0.482
Volume of cones .....	1.55 cm. <sup>3</sup>	1.42 cm. <sup>3</sup>	0.65 cm. <sup>3</sup>	0.66 cm. <sup>3</sup>
Value of $l/A$ for cones.....	0.119	0.109	0.198	0.215
Total volume of vessel .....	88.97 cm. <sup>3</sup>		85.50 cm. <sup>3</sup>	
$l/A$ for whole vessel .....	1.943		7.03	

After measuring the permeability to air for this minimum pore-space, the tube was connected with a water-pump and exhausted. Air-free distilled water was allowed to enter and remain at rest for some considerable time to dissolve any air that had escaped removal by pumping.

The permeability to water was then measured by observing the rate of flow through the column under a measured pressure of water.

TABLE IX.

*Preliminary measurements of the Permeability of Bead Sample D in an unlined tube.*

$S$	$k = \frac{1-S}{B^2}$	$\eta_a P_a \times 10^3$	$\eta_w P_w \times 10^3$	$\eta_a P_a \cdot k$ $d^2$
0.379	26.06	0.780		19.97
0.3695	26.39	.746		19.34
0.366	27.28	.7075		18.95
0.364	27.79	.682	0.515	18.62

§ 21. The disagreement between  $\eta_a P_a$  and  $\eta_w P_w$  in the last line of Table IX is too great to be accounted for by ordinary experimental



errors and was eventually found to be due to imperfect removal of the air from between the interstices of the beads. In subsequent experiments special precautions were taken to ensure the complete removal of the air and the disagreement practically vanished.

More difficult of explanation however were the values of  $\frac{\eta_a P_a}{d^2} \cdot k$  given in the last column; these should, from Slichter's calculation (see § 3), be equal to 10.2. The high permeability observed might, it was thought, be due to the channels, of a larger area than those between the beads themselves, existing all round the inner surface of the tube (Fig. 6). The error due to this cause will depend on the relative diameters of the beads and the containing tube. Where the diameter of a bead is one per cent. of that of the tube a simple calculation shows that the permeability will be increased by about twenty-eight per cent., if the beads are packed in the closest possible manner; for ordinary packing the error will be diminished to about eight per cent.

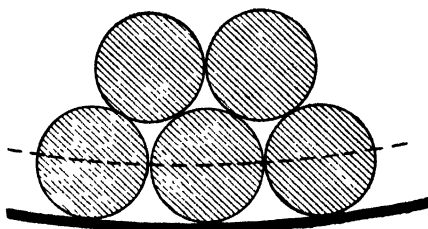


FIG. 6.

But the want of concordance with Slichter's formula cannot be accounted for in this way.

§ 22. If the wall of the tube could be covered with hemispheres fitting closely together, this correction would be practically eliminated; this condition was attained by the following indirect device.

The inner surface was lined with a layer of cement (Chatterton's compound) approximately calculated to be sufficient to embed the beads to half their diameter. In order to obtain an even coating the cement was dissolved in chloroform and poured into the vessel and the chloroform completely evaporated by blowing air through the tube while the latter was continuously rotated in a horizontal position. The tube was then filled with beads and gently warmed to melt the cement into which a layer of beads sank and was firmly held on cooling.

The cement and half of the embedded beads were considered as belonging to the wall and the other half of the glass beads to the main

body whose permeability and pore-space were to be measured. In calculating the latter, half the weight of these embedded beads was added to the weight of the loose beads with which the vessel was filled.

The "effective" volume and area of the tube were obtained from appropriate weighings of the attached cement and beads. In one case the following measurements were obtained :

*Unlined tube*: original volume = 89.0 c.c.;  $\frac{l}{A} = 1.943$ .

The inner surface was then lined with cement (cones excepted). 5.80 grams of beads—diameter = 0.319 mm., density = 2.75—attached themselves and occupied a volume of 2.10 c.c.

*Lined tube*: volume (determined by water content) = 85.15 c.c.

half-volume of beads in lining = 1.05 c.c.

"effective" volume = 86.20 c.c.

volume of cones (from previous measurement) = 2.97 c.c.

„ cylinder (by difference) = 83.23 c.c.

length „ = 12.04 cm.

therefore area of lined part of cylinder = 6.910 sq. cm.

and  $\frac{l}{A}$  for „ „ „ = 1.744

but  $\frac{l}{A}$  for cones (from previous measurement) = .228

therefore "effective"  $\frac{l}{A}$  for whole vessel = 1.972

§ 23. Table X gives the results of the first experiments carried out with the tube lined as described above; and in Fig. 7 curves are drawn comparing the unlined and lined tubes filled with the same beads.

TABLE X.

*Measurements of the Permeability of Bead Sample D in a lined tube.*

$S$	$k = \frac{1-S}{R^2}$	$\eta_a P_a \times 10^3$	$\eta_w P_w \times 10^3$	$\frac{\eta_a P_a}{d^2} \cdot k$
0.3895	22.19	0.901		19.63
0.3835	23.39	.851		19.55
0.3835 (?)	23.39	.811		18.62 (?)
0.373	25.62	.736		18.51
0.3625	28.17	.626	0.611	17.31

Whilst attempting to duplicate these measurements the glass tube broke and it was at this stage that the second vessel was constructed and brought into use. It was also lined with cement and a series of readings obtained with it, for the same size of beads is included in Table XI and in Fig. 7.

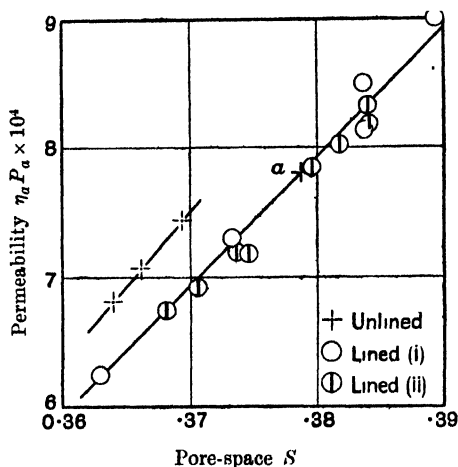


FIG. 7. Comparison of the permeabilities of beads (sample D) in unlined and lined tubes.

It will be seen that while the two lined vessels give results agreeing within about one per cent., the beads in the unlined tube had a permeability some seven per cent. higher. This agrees with the estimate previously given (§ 21).

The highest pore-space which we attempted to work with in the unlined tube gave a point, marked (a), considerably below the true curve; obviously the slight vibration due to unavoidable handling of the apparatus further compacted the beads and the reading must therefore be discarded. With the lined tubes there was naturally much less tendency for this to occur.

#### *Results obtained with Glass Beads.*

§ 24. Series of measurements were now made with five sizes of the carefully graded glass beads, the glass tube being in each case lined with some of the same size beads embedded in cement. After the permeability to air was observed for several degrees of compactness, then water was admitted as described and a reading of the permeability to water obtained.

TABLE XI.

*Summary of the Permeabilities of Glass Beads, of various grades, as measured in lined tubes.*

Diameter of "beads"	Pore-space	Intrinsic Permeability		$\frac{\eta P}{d^2} \times 10^3$	$\frac{\eta P}{d^2} \cdot k$
		to air	to water		
$d$	$S$	$\eta_a P_a \times 10^4$	$\eta_w P_w \times 10^3$		
<i>A</i> 0.938 mm.	0.391	6.76		7.68	17.10
	.370	5.21		5.92	15.54
	.3675	5.125		5.82	15.65
	.3635	4.96		5.63	15.69
			4.70	5.33	14.90
<i>B</i> 0.709 mm.	0.400	4.685		9.31	18.90
	.3925	4.19		8.33	18.02
	.388	3.93		7.80	17.52
	.384	3.806		7.56	17.61
	.373	3.264	3.33	6.48	16.58
				6.63	16.93
<i>C</i> 0.497 mm.	0.376	1.776		7.18	17.89
	.373	1.677		6.77	17.33
	.366	1.538		6.22	16.96
	.366	1.564		6.34	17.28
	.361	1.489	1.523	6.02	17.18
				6.15	17.57
<i>D</i> 0.319 mm.	0.3895	0.901		8.85	19.63
	.3835	.851		8.35	18.62
	.373	.736		7.22	18.51
	.3625	.626		6.15	17.31
			0.611	6.00	16.90
	0.384	0.831		8.15	18.98
	.3795	.785		7.71	18.65
	.3745	.719		7.06	17.83
	.3705	.692		6.795	17.77
			0.6645	6.52	17.05
<i>E</i> 0.250 mm.	0.382	0.802		7.875	18.65
	.3735	.7195		7.065	18.00
	.368	.676		6.63	17.73
			0.655	6.43	17.40
<i>E</i> 0.250 mm.	0.3905	0.516		8.25	18.30
	.384	.470		7.525	17.52
	.379	.4385		7.02	17.05
	.373	.4135		6.62	16.94
	.370	.4035		6.45	16.94
	.370	.410		6.56	17.03
	.366	.377		6.03	16.45
			0.373	5.97	16.27

§ 25. The summarized results are given in Table XI and Fig. 10 and a satisfactory basis for discussing the accuracy of Slichter's calculations for spherical particles of uniform size within the range of the diameters available. The values of  $k$  used throughout this paper are those taken from his formulae (see Table I).

*Results obtained with Sands.*

§ 26. It was decided to extend the investigation to ordinary sand grains also of uniform size. The quartz sand utilized was but slightly water-worn and, after being chemically cleaned, three grades were separated by sifting and elutriation by the same apparatus and in the same manner as the glass beads.

The accompanying photographs enable their shape, size and uniformity to be examined and compared. The smaller grades originally contained much ilmenite, the bulk of which however was removed by careful "panning off."

§ 27. The average diameter of each grade was determined in the usual way by counting out several thousand grains and weighing them. Much greater deviations from the mean were found than with the glass spherical beads, possibly due in part to the variations in composition and consequently of specific gravity.

The actual weighings were as follow:—

TABLE XII.

*Diameter and Specific Gravity of Sand Grains.*

Number weighed	Coarse Sand		Medium Sand	Fine Sand
	1000		500	500
Weighings .....	0.7364	0.7251	0.0170	0.0045
	.7261	.7883		.0045
	.6933	.9207		.0045
	.7405	.8226		.0043
	.7415	.9076		.0045
Average weight per thousand	0.7802 gram.		0.0337 gram.	0.00892 gram.
Specific Gravity .....	2.654		2.653	2.648
Average Diameter .....	0.825 mm.		0.289 mm.	0.186 mm.

§ 28. The same apparatus was used for measuring the permeabilities of the quartz sands to both air and water as had previously been used

for the glass beads. It was considered unnecessary to line the vessel with cement and a layer of the sand on account of the irregular and angular shapes of the individual grains. The results justified this decision.

TABLE XIII.  
*Summary of Permeabilities of Quartz Sands.*

Diameter of Grains	Pore-space	Intrinsic Permeability		$\frac{\eta P}{d^2} \times 10^4$	$\frac{\eta P}{d^2} \cdot k$
		to air	to water		
$d$	$S$	$\eta_a P_a \times 10^3$	$\eta_w P_w \times 10^3$		
0.825 mm.	0.386	3.186	2.026	4.68	10.71
	.378	3.018		4.43	10.84
	.3695	2.706		3.975	10.48
	.361	2.545		3.74	10.68
	.3485	2.144		3.155	10.12
	.347	2.070		3.04	9.89
				2.97	9.68
0.289 mm.	0.412	0.447	0.291	5.35	9.87
	.393	.3640		4.36	9.40
	.385	.3265		3.91	9.03
	.381	3065		3.67	8.76
	.378	.2955		3.535	8.66
	0.408	0.4525		5.42	10.33
	.395	.383		4.585	10.08
	0.403	0.4005		4.795	9.51
	.385	.3385		4.055	9.36
	0.401	0.3885		4.65	9.37
	.373	.294		3.525	9.02
				3.525	9.02
	0.435	0.2525		7.30	11.20
	.4045	.1820		5.265	10.33
	.3945	1650		4.78	10.17
	.377	.1320		3.82	9.44
				3.74	9.22
0.186 mm.			0.1290		

*Discussion of results.*

§ 29. The justifiability of Slichter's assumptions and calculations may be judged by the agreement of these results with the formula

$$\eta P = 10.2 \frac{d^2}{k}.$$

*I.e.* the expression  $\frac{\eta P k}{d^2}$  should be equal to 10.2 for all sizes of particles and for each arrangement of pore-space.

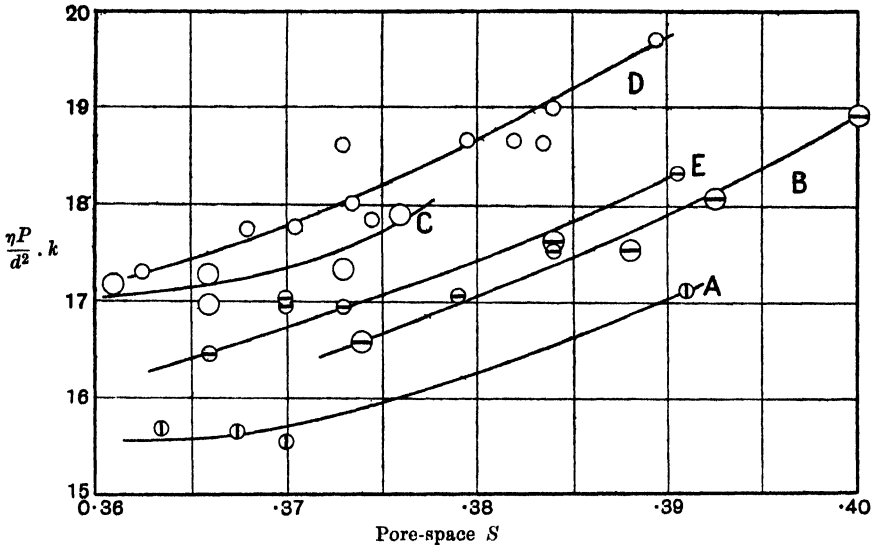


FIG. 10. Summary of results with glass beads (see Table XI).

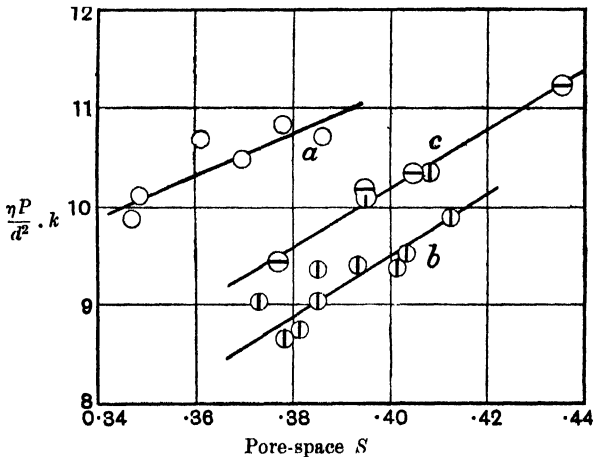


FIG. 11. Summary of results with sands (see Table XIII).

§ 30. It will be observed that for the glass beads however no such agreement is to be found, for the value obtained varies from 15.5 to 19, and increases with increase of pore-space. In other words, the permeabilities both to air and water are from 50 to 85 per cent. greater than Slichter's calculated values.

The explanation of this is almost certainly to be found in his method of considering each soil capillary as if it were a double triangular-shaped pore with a *partition down the centre* instead of as an *undivided* more or less rhomboidal pore at its narrowest part. It is obvious that this assumption must considerably undervalue the permeability of the pore,

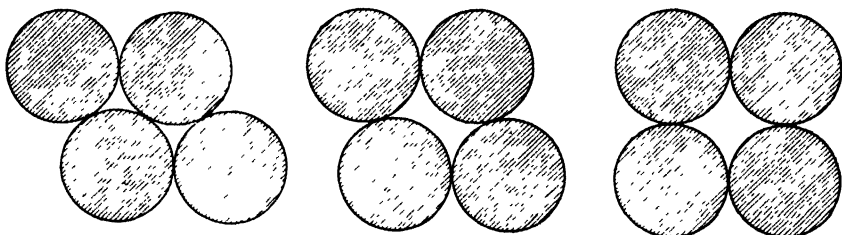


FIG. 12.

but it would be difficult to make anything like an exact estimate of the deficiency. Any such correction should vanish when the angle of packing is sixty degrees, for, as the pore-space approaches the minimum possible, so does the pore actually become divided into two and Slichter's method of discussion would then hold good. [See Fig. 12.]

An examination of the graph (Fig. 10) bears this out, for if extrapolated to the minimum pore-space the value of  $\frac{\eta P k}{d^2}$  evidently will in general approximate to the calculated figure—10.2.

§ 31. On the other hand, the experiments with ordinary sands, though showing a rather large percentage error, give an average result of 9.45 for the permeability to air ( $\eta_a P_a$ ) and 9.31 for the permeability to water ( $\eta_w P_w$ ). This is, considering the difficulties of accurate measurement, a satisfactory concordance.

The obvious explanation (of this less perfect material agreeing more perfectly with the theoretical formula) is that the angular shapes of the particles do practically have the effect of dividing the pore into two triangular passages as assumed in the formula.

### Conclusion.

§ 32. As the particles in ordinary soils are not perfect spheres but more or less angular in shape, the experiments described in this paper show that the formula  $\eta P = 10.2 \frac{d^2}{k}$  holds quantitatively for variations of the pore-space and of the diameters of the soil particles. This will be so



whether the permeating fluid be *air or water, provided that the actual sizes of the soil particles are unaffected by the presence of water*<sup>1</sup>.

With this factor taken into account it is therefore legitimate to consider a soil as statistically composed of a bundle of capillary tubes when discussing the movements of air and water through it.

In conclusion we have to acknowledge our indebtedness to Professor T. R. Lyle for valuable advice and suggestions, to Mr H. J. Grayson for assistance in preparing the micro-photographs and to the Victorian Government for financial assistance towards the expenses of this research.

<sup>1</sup> It was pointed out in Part I. of these researches (*loc. cit.* §§ 9, 32) that the behaviour with water is a most important property of the soil; for whereas with clean sands the ratio  $\frac{\eta_a P_a}{\eta_w P_w}$  will be but slightly greater than unity, the amount of colloidal matter present will cause a corresponding increase in its magnitude.

# INVESTIGATIONS ON "SICKNESS" IN SOIL.

## I. SEWAGE SICKNESS.

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THE experiments of Russell and Hutchinson<sup>1</sup> have shown that the micro-organic population of ordinary soils is not working at a maximum efficiency; there exists a biological factor, provisionally identified with the soil protozoa, detrimental to bacteria and limiting their numbers and activities. It follows that any change in the conditions of the soil that is more favourable to the harmful organisms than to the bacteria will disturb the normal equilibrium between these two sets of organisms and lead to a relative reduction in bacterial numbers and activity. We shall therefore expect to find that causes not in themselves harmful to bacteria may bring about a reduction of bacteria through favouring the development of the detrimental factor.

About three years ago the soil of the Kegworth sewage farm, in which one of us is interested, showed marked signs of "sewage sickness." When the sewage was run on to the land it no longer percolated as rapidly as before or gave rise to so pure an effluent: instead it lay about in pools and the effluent was unsatisfactory. It was no longer possible to apply sewage while the crops were growing as is done on a larger farm not far away: when this was attempted with mangolds or cabbage the leaves began to fall and the plants were finally killed. On going over the ground together and taking careful note of the phenomena it seemed that we had here a case where the

<sup>1</sup> This *Journal*, 1909, 3, 111.

state of affairs mentioned above is realised, and the bacterial efficiency was being kept down by excess of a detrimental factor; we therefore decided to investigate the problem from this point of view. The Kegworth Parish Council kindly gave us the necessary permission, allowed us access to the farm at all times, and granted us facilities for carrying out our experiments, for which we desire to tender to them our very sincere thanks.

The land treatment of sewage consists in allowing the sewage to run on to the land with or without a preliminary treatment. At Kegworth the sewage is simply screened and then applied to land that has been ploughed and is later to be cropped; elsewhere it is applied direct to growing crops. So long as percolation is rapid the method works well, but it breaks down, and "sewage sickness" sets in, directly percolation becomes too slow. So real is the danger that only particular types of soil are suitable for sewage farming, a light loam lying on a sand being perhaps one of the best<sup>1</sup>. Indeed the chief point in the management of a sewage farm seems to be to arrange the drainage system, the distribution of the sewage and the cropping in such a manner as to prevent the land from becoming waterlogged. Thus at the Aldershot camp sewage farm, where, Col. Jones<sup>2</sup> informs us, sewage sickness is unknown, the sewage is applied direct to rye grass immediately after a crop has been taken and is run on till the land is sufficiently wet or till the crop has grown too tall, when no more is applied till another crop is cut. It is also run on to land carrying mangolds. The third crop, oats, however, receives no sewage. When we saw the crops their healthy condition was sufficient proof of the absence of waterlogging.

Examination of the sewage sick field showed that the falling off in the rate of percolation was due to at least two causes. Wherever a pool of sewage had stood we found a greenish-black slime, black below the water-level and green at and above it, which under the microscope was seen to contain living organisms—algae, *euglena* and numerous others. The black material also penetrated into the soil for a short distance. A second cause is the deflocculation of the clay by the free

<sup>1</sup> Such a soil may take 30,000 gallons of sewage per acre per day, equivalent to nearly 1½ inches of rainfall. Other land, however, may take only 3000. At Birmingham the average quantity has been 6000 (Watson, J. D., *Proc. Instit. Civil Engineers*, 1910, CLXXXI, Part III).

<sup>2</sup> The details of Col. Jones' management are described in *Natural and Artificial Sewage Treatment*: Col. A. S. Jones and H. A. Roechling, London, 1902. The land treatment of sewage is also fully discussed in the *5th Report of the Sewage Commission*.

alkali of the sewage<sup>1</sup>. Both these causes render the soil sticky and impermeable. They can, however, be put out of action: the deflocculation by dressings of lime, and the accumulation of black slimy material by ploughing up the land into ridges and allowing it to dry. Both devices are successfully adopted in sewage farming.

But there is another factor involved both in the wet soil and in the drier and better aerated "rested" soil. Our experiments show that in no case is the bacterial efficiency as high as might be expected. Counts made by the gelatine plate method showed that some 20 to 30 millions of bacteria were commonly present per gram of wet sewage-sick soil, and no marked rise set in when the moisture was reduced and the air supply increased to what might have appeared more favourable proportions. Considering the large amount of easily decomposable nitrogenous organic matter present, and the enormous stimulus such compounds afford to the multiplication of bacteria, these numbers must be regarded as distinctly low; the poor arable soils investigated by Russell and Hutchinson contained 10 to 15 millions per gram, which numbers ran up to 150 millions per gram after addition of 0.1 per cent. of peptone. It thus appears that some factor is keeping down the bacterial population in a sewage-sick soil even under favourable aeration conditions.

This detrimental factor is, however, put out of action by treatment with small quantities of antiseptics (toluene and carbon disulphide were used in the experiments) and the bacterial numbers then rise rapidly to between one and four hundred millions per gram; a great increase in the rate of ammonia production also takes place (Tables II and III). We know of no ordinary soils where partial sterilisation produces anything like so marked an effect; it is clear that we are dealing with a detrimental factor of unusual potency.

It was soon found that the effect of partial sterilisation was an improvement in the soil as a medium for bacterial activity, and not an improvement in the bacterial flora. Indeed the new flora proved to be actually less vigorous than the old when both were placed under similar conditions; for example, it did not attain to such high numbers or bring about so much decomposition as the old flora introduced into the partially sterilised soils and therefore living in equally favourable

<sup>1</sup> In the present case the sewage is mainly domestic, there being no trade effluent except from one large brewery.

The question is more fully discussed by us in *Journ. Soc. Chem. Ind.* 1911, **30**, 471, "Sewage sickness in soil and its amelioration by partial sterilisation."

circumstances (Table V). Thus in one experiment the untreated soil contained 31 million bacteria per gram, the soil treated with carbon disulphide contained 110 millions per gram, but the same treated soil to which the original flora had been introduced by inoculation with 0·5 per cent. of the untreated soil contained 475 millions; now that the bacteria of the original soil have as good a chance of multiplication as those of the partially sterilised soil they increased to a markedly higher extent.

The unsuitability of the untreated "sick" soil for bacterial growth is not due to an accumulation of toxic substances by bacteria because there is no falling off in numbers on the partially sterilised soils even after three months in spite of their very high bacterial content, so long of course as the soil does not become too dry. Further, the very susceptible nitrifying organisms worked quite well in the untreated soil, and they would hardly have done so in presence of a toxin.

The addition of an aqueous extract of untreated sick soil to partially sterilised soil had no adverse effect on the growth of the bacteria or on the amount of decomposition (Table VI). The harmful factor is thus not carried by a water extract; it is neither a soluble toxin nor bacteria.

When, however, some of the untreated soil was added to the partially sterilised soil there was a large reduction in bacterial numbers, especially in the active forms, and the rate of decomposition fell off considerably. The reduction did not set in at once but took some weeks to manifest itself (Table VI). Thus the harmful factor is slowly transferred to the partially sterilised soil on contact with the untreated soil. It shows, in fact, the phenomena of growth and is therefore presumably biological in character.

These phenomena are in complete agreement on all points with those observed on normal soils, and the conclusion seems irresistible that the same detrimental factor comes into play in both cases, but its action is intensified in the sewage-sick soils. Reference to the paper by Russell and Hutchinson will show the reasons for considering the harmful factor in ordinary soils to consist in amoebae and other protozoan organisms. Our experiments with sewage-sick soils lead us to conclude (1) that a factor detrimental to bacteria occurs in sewage-sick soils and is precisely similar in nature to that present in ordinary soils, (2) the detrimental factor occurs to an abnormal extent in the sewage soil, and therefore appears to be much favoured by the mixture of water and organic matter that constitutes sewage, this being the condition that brings on sewage sickness, (3) one of the reasons for

sewage sickness is the reduced bacterial efficiency consequent on the abnormal development of this injurious factor. These conclusions, it will be observed, are statements of experimental facts and are independent of any hypothesis as to the nature of the injurious factor, but they accord extremely well with the protozoan hypothesis, which was based on a wholly different set of considerations. •

The difficulty in the way of finding out precisely which are the harmful organisms in a normal soil is much increased when we pass on to sewage-sick soils. The flora and fauna are very extensive and have not been adequately explored, although it is clear that they would repay investigation from the very interesting work of Fowler<sup>1</sup> and of Meixner<sup>2</sup> on the effluents from bacterial beds. Some of the forms are no doubt helpful in the decomposition process, but it is a simple matter to demonstrate the presence of amoebae and other protozoa known to feed on bacteria, and even to separate living forms from the sewage soil by proper centrifuging.

Whichever the harmful organisms may be, they are much more susceptible to the effects of antiseptics than are bacterial spores, and are readily killed by toluene, carbon disulphide, or heat. When that has been done the bacteria, as we have seen, multiply more freely and effect a greater amount of decomposition, and the soil regains or more than regains its original efficiency as a purifying agent. A number of experimental filters made of sewage-sick soil were set up equal in every respect and equally dosed with sewage, but differing in that some were partially sterilised while others were not. In all cases the effluents from the partially sterilised filters were decidedly better than those from the untreated filters, indicating a higher degree of purification (Tables VII and VIII). This conclusion was confirmed by treating a large area of land with toluene, laying drains and comparing the drainage water with that from a similar but untreated area. Again it was found that the purification was more complete after partial sterilisation.

Thus it appears that no complicating factor comes into play when our laboratory results are applied to field conditions, and we may draw the practical conclusion that the biological factor detrimental to bacteria flourishes in sewage treated soils and reduces the efficiency of the soil

<sup>1</sup> Fowler, G. J., *City of Manchester Rivers Dept. Annual Report*, 1911. We understand that Dr Fowler and Mr Crabtree are extending these interesting observations.

<sup>2</sup> Meixner, A., "The fauna of the Bradford coke bed effluent" (*Proc. Camb. Phil. Soc.* 1908, **14**, 530).

as a purifying agent. The physical factors in sewage sickness may be overcome by methods already in use: the deflocculation by addition of lime or chalk, and the formation of slime on the surface by deep ploughing and exposing the land in high ridges to dry. Both methods are recognised features of good sewage farm practice. But neither of them kills the factor injurious to bacteria, for we found it to persist in our laboratory experiments where the texture of the soil, the aeration and the water supply were all that could be desired; indeed from our results we should expect to find it in all sewage farm soils. In order to kill this factor and to secure maximum bacterial efficiency it is necessary in addition partially to sterilise the soil either by heat or by antiseptics. Paring and burning proved to be an efficient method of heating the soil, but the application of antiseptics on the large scale presents difficulties that have not yet been overcome. Both toluene<sup>1</sup> and carbon disulphide can be obtained cheaply, but it is not necessary to restrict attention to these; any antiseptic may be expected to serve provided it is volatile or decomposes with formation of innocuous compounds when its work is done. Experiments with various other antiseptics are in hand in connexion with the partial sterilisation of horticultural soils, and there is every reason to suppose that any method suitable for these would also prove suitable for sewage-sick soils.

### *Experimental.*

*Analysis of the soil.* The soil was taken from a field that had been receiving sewage for some months past and was about to lie up for a time. It was strongly alkaline to litmus paper and coated in places just above the little pools with a dark green slimy matter and in places where water had lain with a black deposit that penetrated a little way into the soil. Its composition on different dates is given in Table I.

In the wet winter samples the amount of ammonia is extraordinarily high in comparison with the 1 or 2 parts per million found in ordinary arable soils. In the drier May sample the ammonia is lower but the nitrates have now risen considerably.

The ammonia is estimated by distillation *in vacuo* with magnesia<sup>2</sup> and the "nitrate, etc." by extraction with water, boiling off all ammonia from the extract after addition of magnesia, acidifying with acetic acid, reducing with a zinc-copper couple and estimating the ammonia thus

<sup>1</sup> We are now paying 10d. a gallon for commercial "toluole."

<sup>2</sup> Russell, this *Journal*, 1910, 3, 233.

formed. The qualification "etc." is added because we find that rich soils such as our sewage soil yield up to water other reducible and non-volatile compounds besides nitrates, so that the results always come out too high. For our present purpose, however, the total reducible nitrogen compounds give a better measure of the amount of decomposition than the nitrates alone.

TABLE I. *Analysis of the sewage-sick soil.*

	Nov. 20, 1910	Jan. 5, 1911	Feb. 10, 1911	May 24, 1911
Moisture present, per cent. ....	36.6	38.3	31.6	20.6
Loss on ignition, per cent. ....		12.7		
<i>Composition of dry matter</i>				
Carbonates (as $\text{Ca}(\text{O}_3)_2$ , per cent. ....		0.6		
Total nitrogen, per cent. ....		0.41		
Nitrogen as ammonia, per million .....	180	150	100	24
,,    ,, nitrate, per million .....	10	16	31	134
Bacteria present, millions per gram of moist soil		37 <sup>1</sup>	21 <sup>1</sup>	

In order to make the bacterial counts about 10 grams of soil were placed in a weighing-bottle and weighed, then tilted into a graduated litre flask containing about 750 c.c. of sterile distilled water. The flask was corked and shaken for half an hour, sterile water was added to the litre mark and the whole shaken again. 1 c.c. was then transferred to 100 c.c. sterile distilled water and the mixture was agitated for three or four minutes, 1 c.c. of this was transferred to another 100 c.c. sterile distilled water and shaken, finally 1 c.c. of this last mixture was added to the gelatine and poured on to the plates. Instead, however, of the ordinary Petri dish we used the special form one of us<sup>2</sup> had for some time adopted for bacteriological work. The method is not capable of great refinement and duplicate counts do not agree very closely, but the differences obtained in our experiments are far beyond the errors of the determination.

We have not studied the bacterial flora in any detail, but judging from the appearance of the colonies it did not appear to be very varied

<sup>1</sup> Owing to the very great difficulty of sampling, these numbers are necessarily only approximate.

<sup>2</sup> Golding, "A New Bottle for Cultures," *Journal of the Society of Chemical Industry*, 1906, **25**, p. 677.



in any of the soils. From the soil treated with carbon disulphide (Table II) we obtained numerous little matted colonies forming a firm pellicle difficult to break with a platinum needle: these on further examination were found to consist of long chains or threads of the leptothrix type not motile on gelatine but somewhat motile on agar. From the tolued soil (Table II) a number of thinnish grey colonies with darker centres were obtained, while the reinfected tolued soil (Table V) yielded in addition a number of smooth yellow colonies. The brown forms, some of which were threads, varied in their occurrence; more were found in the stored soil (Table III) than in any other samples; all were killed by heat and carbon disulphide, some, however, escaped the action of toluene in the Table III series but not in the rather different Table II series. There were remarkably few liquefying organisms in any of the samples.

Moulds developed freely in the heated soils but not in the heated and limed soils (Table III).

*The effect of partial sterilisation on bacterial activity.* The soil as it came to the laboratory was much too wet for experimental purposes; it was therefore allowed partially to dry, passed through a 3 mm. sieve and then divided up into a number of equal portions of 500 grams each. These were put into wide-mouthed bottles plugged with sterile cotton-wool and subjected to their appropriate treatment: some were heated to 98° C. for three hours, while others received 2 per cent. of carbon disulphide or toluene and after two days were spread out at ordinary temperature till the antiseptic had completely evaporated; water was then added to bring the soil to the proper state of moistness.

In the experiments recorded in Table II the soil was treated as soon as possible after it came from the farm. Dealing first with the January 5 sample: a comparison of the data for the untreated soil as it was freshly taken (Table I) with the results given here shows that the removal of the excess of water has remarkably little effect on the bacterial numbers or on the amount of decomposition. There is a considerable immediate loss of ammonia—from 150 parts per million in the wet soil to 70 parts in the drier soil—but very little change took place in the untreated soil after the ninth day in spite of the very favourable temperature, moisture and aeration conditions. Bacterial multiplication and the production of ammonia are both more rapid and carried to a further extent in the soil treated with carbon disulphide.

The most rapid decomposition, however, is in the heated soil, but

TABLE II. *Influence of partial sterilisation on bacterial activity in sewage-sick soils fresh from farm.*

(a) Sample taken January 5, 1911. Moisture reduced to 12 per cent.

	Bacteria present per gram of dry soil, millions			Nitrogen present as ammonia, parts per million of dry soil		Nitrogen present as nitrate, parts per million of dry soil		Sum of ammonia + nitrate, parts per million of dry soil	
	At start Jan. 5	After 9 days	After 22 days	After 9 days	After 22 days	After 9 days	After 22 days	After 9 days	After 22 days
Untreated soil . . .	37 <sup>1</sup>	31	31	74	91	12	9	86	100
Heated to 98° . . .		22	28	128	257	12	13	140	270
Treated with CS <sub>2</sub> . .		130	110	93	139	16	8	109	147

<sup>1</sup> On wet soil, 41 per cent. of water being present.(b) Sample taken May 24, 1911. Moisture reduced to 16 per cent.<sup>2</sup>

	Bacteria present per gram of dry soil, millions			Ammonia present, parts per million of dry soil			Nitrate present, parts per million of dry soil		
	After 2 days	After 15 days	After 4 months	After 2 days	After 15 days	After 4 months	After 2 days	After 15 days	After 4 months
Untreated soil . . . . .	Plates spoiled by hot weather	44	43	25	25	12	140	191	327
Heated to 98° . . . . .		34	73.5	35	233	378	109	108	150
Treated with toluol . .		222	272	76	210	277	84	85	157

<sup>2</sup> At 16 per cent. of moisture the soil is in a very nice moist condition eminently suited for the growth of plants.

	Ammonia + nitrate present, parts per million of dry soil		
	After 2 days	After 15 days	After 4 months
Untreated soil . . . . .	165	216	339
Heated to 98° . . . . .	144	341	528
Treated with toluol . .	160	295	434

here the bacteria only multiply very slowly and the flora is greatly restricted, only very few species showing on the plates. This is the usual course of events on heated soils and indicates some fundamental difference between the effect of heat and of antiseptics that we have not yet investigated. We know, however, that a chemical change is brought about by the heat because the soil takes on a characteristic odour, yields a darker coloured aqueous extract and becomes specially favourable for the development of moulds and unfavourable for the development of the sensitive nitrifying organisms<sup>1</sup>. A change in physical properties is also brought about by heat.

The May samples show the same general relationships; the first bacterial counts were lost by the liquefaction of the plates during a sudden spell of very hot weather, but subsequent counts show very little change in numbers in the untreated soil; there is, however, a steady and continuous decomposition, which is clearly normal and unaffected by any circumstance more harmful to the sensitive nitrifying organisms than to the others because nitrification is proceeding more rapidly than ammonia production. Toluene treatment leads to a great rise in bacterial numbers and a considerable increase in the amount of decomposition. Heat, as before, causes the greatest increase in the rate of ammonia production but no increase in bacterial numbers in the early stages. Later on, however, the bacteria rise to an enormous extent, but the flora is still very restricted and there is a considerable development of mould.

The experiments recorded in Table III were designed to ascertain whether the harmful factor is removed by treatment with lime or by long exposure to air. The soil was partially dried to reduce the moisture to 16 per cent. and then kept for seven weeks (from June 23 to August 10, 1910) under very favourable conditions of aeration, moisture and temperature; it was then subdivided and treated as before. An additional set was introduced here in which the soil was heated to a temperature sufficiently high to cause a certain amount of charring. The results are of the same kind as when the soil is freshly treated and show that the injurious factor is not removed by aeration or by lime.

In this case initial bacterial counts were taken throughout, and they show as usual that partial sterilisation much reduces the numbers, heat being particularly drastic in its effects. In the soils treated with toluene and carbon disulphide the bacteria rapidly multiply to a marked

<sup>1</sup> See also Russell and Hutchinson, this *Journal*, 1909, **3**, 111.

TABLE III. *Influence of partial sterilisation on sewage-sick soils that have been maintained for some weeks under favourable conditions of aeration, temperature, etc.*

Number of bacteria, millions per gram of dry soil. Moisture reduced to 16 per cent.

	No lime applied				Lime applied (0.2 per cent.)			
	At start, Aug. 11, 1910	Aug. 24	Oct. 19	Nov. 21	At start, Aug. 11, 1910	Aug. 24	Oct. 19	Nov. 21
Untreated soil .....	22	12	35	91	14.4	29	30	38
Soil heated to 98° C. ....	0.04	18	37	37	0.8	52	53	55
Burnt soil .....	very few	—	29	42	very few	50	54.5	49
Soil treated with carbon disulphide	2.8	11	136	253	1.5	348	355	168*
Soil treated with toluene ....	4.2	24	60	441	1.8	161	199	229

Nitrogen present as ammonia, parts per million of dry soil

	No lime added				Lime added			
	At start, Aug. 12	Aug. 24	Oct. 19	Nov. 21	At start, Aug. 12	Aug. 24	Oct. 19	Nov. 21
Untreated soil .....	70	50	16	8	70	42	13	16
Soil heated to 98° C. ....	80	161	345	376	80	198	325	342
Burnt soil .....	—	193	204	220	—	159	271	217
Soil treated with CS <sub>2</sub> .....	75	167	228	205	75	157	229	134*
Soil treated with toluene ...	75	154	103	94	75	171	98	57

Nitrogen present as nitrate, etc., parts per million of dry soil

Untreated soil .....	327	346	417	440	327	389	460	435
Soil heated to 98° C. ....	332	332	335	350	338	346	336	206
Burnt soil .....	—	43	24	52	—	51	37	74
Soil treated with CS <sub>2</sub> .....	294	306	305	318	316	306	319	317*
Soil treated with toluene ...	281	289	447	426	309	319	450	500

Sum of ammonia + nitrate, etc., formed, parts per million of dry soil

	No lime added				Lime added			
	Already present at start, Aug. 12	Additional amount formed after			Already present at start, Aug. 12	Additional amount formed after		
		13 days Aug. 24	69 days Oct. 19	102 days Nov. 21		13 days Aug. 24	69 days Oct. 19	102 days Nov. 21
Untreated soil .....	397	0	37	55	397	34	66	53
Soil heated to 98° C. ....	412	81	268	314	418	126	243	130
Burnt soil .....	236	0	0	35	210	0	100	80
Soil treated with carbon disulphide	370	103	163	153	391	72	158	60*
Soil treated with toluene ....	356	87	194	165	384	106	164	174

\* This sample had become very dry during the last four weeks of the experiment.

extent and maintain their high numbers without any sign of falling off; there is also a great increase in the rate of ammonia production. The heated soil as usual behaves differently and throughout the whole of the time only contains a low number of bacteria, although the amount of decomposition is higher than in any other case.

Lime has, at first, a strong depressing action on the bacteria, reducing the numbers in the untreated soil from 22 to 14 millions and in the chemically treated soils from 2·8 and 4·2 to 1·5 and 1·8 respectively. In a very short time, however, the numbers rise, particularly in the soils treated with carbon disulphide and toluene, and the amount of nitrate also increases; neither of these effects is permanent. The effect on the bacteria in the heated soil is quite distinct, but it is so small as to rule out any hypothesis that the unsuitability of the heated soil for bacterial growth is due to the formation of acid substances by heat. The action on the untreated soil is very small and clearly demonstrates that lime does not put out of action the harmful factor, although it does somewhat hasten the decomposition.

There is a distinct qualitative difference between the action of carbon disulphide and that of toluene. Carbon disulphide is the more effective antiseptic and not only reduces the bacterial numbers to a greater extent but even kills certain forms—notably the brown ones—that are partially spared by the toluene. It seems likely on other grounds that the difference in behaviour is due to physical causes; the vapour of carbon disulphide has great power of penetrating the soil and of getting at the organisms; while toluene, with its lower power of penetration, leaves some of the organisms untouched.

TABLE IV. *Percentage of nitrogen present in soils 70 days after partial sterilisation (Oct. 19 samples of Table II used).*

	No lime added	Lime added
Untreated soil .....	·561	·570
Soil heated to 98° C. ....	·548	·535
Burnt soil .....	·524	·524
Soil treated with CS <sub>2</sub> .....	·539	·561
Soil treated with toluene...	·541	·541

In consequence two interesting effects are produced in the toluened soil (1) high bacterial numbers (441 millions) are subsequently reached, the soil being really a partially sterilised soil + a trace of untreated soil (see Table V), (2) nitrification sets up after a time, although it is suppressed in all the other partially sterilised soils. This difference between the two antiseptics is not always observed; under other

circumstances, not yet definitely characterised, treatment with toluene kills all the brown forms and leads to no greater bacterial development than treatment with carbon disulphide.

The increased rate of decomposition in the partially sterilised soils causes as usual a loss of nitrogen, and in consequence they contain a smaller percentage of nitrogen after the lapse of some weeks than the untreated soil, as seen in Table IV.

In our experience this relative loss of nitrogen invariably sets in after partial sterilisation.

The comparison of the new flora arising after partial sterilisation with the old is made by introducing a little of the untreated soil (0.5 per cent.) into the partially sterilised soil. In all cases the old flora has the greater power of multiplication and of effecting decomposition, and the results, given in Table V, show beyond doubt that the effect of partial sterilisation is not to improve the bacteria but to give them a better opportunity of working.

TABLE V. *Comparison of the old bacterial flora with the new.*

	Numbers of bacteria, millions per gram			Nitrogen present as ammonia and nitrate, parts per million of dry soil				
	At start, Jan. 5, 1911	Jan. 14	Jan. 27	As ammonia		As nitrate, etc.		Sum of ammonia + nitrate, etc.
				Jan. 14	Jan. 27	Jan. 14	Jan. 27	
Untreated soil . . . . .	37	31	31	73.8	90.8	12.3	9.1	100
Soil treated with CS <sub>2</sub> : new flora . . . . .		130	110	93.2	139	16.3	7.9	147
Soil treated with CS <sub>2</sub> , then reinfected with 0.5% untreated soil: old flora . . . . .		110	475	105	263	11.5	9.2	272

Moisture = 12 per cent.

The unsuitability of the untreated soil for bacterial growth is not due to any substance that can be washed out, for the aqueous extract of the untreated soil has no depressing effect on bacterial activity in the partially sterilised soil. The extract was made by shaking 200 grams of soil with 500 c.c. of water, allowing to settle for an hour and filtering through paper: 30 c.c. were used for each bottle of 500 grams of soil. No substantial difference is produced in the bacterial numbers nor in the rate of decomposition, although there is a change in the course of the reaction due to the introduction of nitrifying organisms (Table VI). On September 30, however, a more complete bacteriological

examination was undertaken and a separate enumeration was made of the forms not killed by toluene, which may perhaps without serious error be regarded as spores. For this purpose 20 grams of soil were allowed to stand in a weighing-bottle with 1 c.c. of toluene for one hour and then left three hours in a vacuum so that the toluene might evaporate; weighings and attenuations were then made and plates poured in the usual way. The results show a surprisingly small number of forms resistant to toluene in the untreated soil, and further examination has shown this to be a usual characteristic of the untreated soil. These forms accumulate to a much greater extent in the partially sterilised soils, where also there occur a far larger number of forms killed by toluene.

A search for plant toxins decomposable on partial sterilisation also led to negative results. Barley seedlings made even better growth in extracts of untreated soil than in extracts of freshly treated soils; the results of a typical experiment were:—

	Untreated soil	Soil heated to 55° C.	Soil heated to 100° C.	Soil treated with toluene
Weight of shoot, grams ...	·079	·076	·061	·054
"    root    "    .....	·020	·018	·014	·011
Total per plant.....	·099	·094	·075	·065

The poorer growth in the extracts of partially sterilised soils is under investigation.

The harmful factor resides in the untreated soil and can be transmitted to the partially sterilised soil. When 5 per cent. of untreated soil is added to the toluened soil the rise in numbers due to the addition of the untreated organisms is followed by a gradual fall both in spores and in active forms, and there is a similar falling off in the rate of decomposition. The process of transmission is slow and somewhat erratic and did not complete itself in any of our experiments, but it went sufficiently far to cause a reduction of 25 per cent. in the bacterial numbers. The results are given in Table VI.

The very close resemblance between these phenomena and those observed with ordinary arable soils shows that the factor injurious to bacteria is of the same nature in both cases. It is considered by Russell and Hutchinson that the active protozoa of ordinary soils constitute the harmful factor there, and examination was therefore

TABLE VI. *Effect of adding untreated soil, and the aqueous extract of untreated soil, to toluened soil. Moisture=17.5 %.*

	Bacterial numbers			Details of Oct. 3 counts	
	After 2 days, May 29, 1911	June 13	Oct. 3	not killed by toluene	killed by toluene
Untreated soil .....	Plates	44.5	43	11	32
Toluened soil .....	spoiled	222	272	161	111
Toluened soil + aqueous extract of untreated soil	by	178	281	44	237
Toluened soil + 5 % untreated soil .....	hot	152	209	117	90
Toluened soil + 10 % untreated soil .....	weather	211	193	101	92

	Nitrogen present as ammonia + nitrate, etc., parts per million of dry soil								
	As ammonia			As nitrate, etc.			As ammonia + nitrate, etc.		
	May 29	June 13	Oct. 3	May 29	June 13	Oct. 3	May 29	June 13	Oct. 3
Untreated soil .....	25	25	12	140	191	327	165	216	339
Toluened soil .....	76	210	277	84	85	157	160	295	434
Toluened soil + aqueous extract of untreated soil .....	92	220	133	82	92	281	174	312	414
Toluened soil + 5 % untreated soil	80	180	8	84	130	210	164	310	218
Toluened soil + 10 % untreated soil .	79	142	7	85	147	316	164	289	353

made for protozoa in sewage-sick soil. They were found in considerable numbers. The usual method of inoculating soil into hay infusion and incubating the mixture fails to discriminate between active forms and cysts. We therefore used a centrifugal method to bring out some of the more active forms. About 10 grams of the soil are well ground in a mortar with 50—100 c.c. of water and the muddy liquid is poured into the cups of the centrifuge, the opposite pairs being balanced by adjusting the quantity of liquid added. The centrifuge is then worked at a fairly high speed till the soil lies at the bottom. The opalescent liquid is next decanted and spun a second time at higher speed; the sediment now sinking to the bottom contains living organisms including many flagellated and other protozoa (some of which are attached to soil particles) and motile bacteria. In examining the sediment under the microscope we obtained the best results by mounting it in a drop of the supernatant liquid. No living forms could be obtained from soil treated with toluene.



The disadvantage of the method is that it reveals only those organisms of the same order of size as bacteria and requires the same power of the microscope in order to be seen. It demonstrates clearly, however, that bacteria are not the only active forms in the soil but that other active organisms also occur<sup>1</sup>.

A detailed study of Tables III and VI shows that losses of ammonia sometimes take place over a long period. This happened in Table III on the untreated limed soil and on several of the partially sterilised soils between the 69th and the 102nd day, also in Table V on the tolunened soil + 5% untreated soil. Some volatilisation of ammonia certainly took place, but we obtained evidence of an actual assimilation of ammonia on the untreated soil whenever large amounts of ammonia were present. This is a very unusual action in our experience, and we have never been able to find it on other soils<sup>2</sup>. No assimilation of nitrates took place. The following experiment shows that only 75 out of the 112 parts of nitrogen added as ammonium sulphate were recovered in the form of nitrate and ammonia, the other 37 having disappeared, while recovery of nitrogen added as nitrate was complete:—

	Nitrogen present as $\text{NH}_3$ , parts per million of dry soil			Nitrogen present as nitrate, parts per million of dry soil			Nitrogen present as $\text{NH}_3$ + nitrate, parts per million of dry soil		
	At start	After 40 days	After 93 days	At start	After 40 days	After 93 days	At start	After 40 days	After 93 days
Untreated soil alone . . . . .	112	136	78	215	338	383	327	474	461
„ + $(\text{NH}_4)_2\text{SO}_4$ . . . . .	215	276	164	224	286	372	439	562	536
„ + $\text{NaNO}_3$ . . . . .	119	181	101	290	391	443	409	572	544

	Amount of added nitrogen recovered as nitrate and ammonia		
	At start	After 40 days	After 93 days
Untreated soil + $(\text{NH}_4)_2\text{SO}_4$ . . . . .	112	88	75
„ „ + $\text{NaNO}_3$ . . . . .	82	98	83

<sup>1</sup> Our best results were obtained with a centrifuge running at 4000 revolutions per minute but we could get satisfactory results even with a Gerber centrifuge in which the butyrometer tubes were replaced by test tubes of stout glass. One of us has used this latter method as a class exercise to demonstrate the presence of motile bacteria and other organisms in the soil.

<sup>2</sup> Cf. Russell and Hutchinson, *op. cit.* Vol. III. p. 129, and Russell and Petherbridge, this volume, p. 96.

It did not appear that this assimilation went on in the partially sterilised soil.

*Practical application of the results.*

The problem we started with was to study the causes of sewage sickness, and our experiments show that they are partly physical and partly biological, and include an abnormal development of a factor harmful to bacteria which is similar in every respect to the factor operating in ordinary soils. This biological factor is therefore not a condition peculiar to sewage treated soils but is of much more general interest as an extreme case of an action going on in all soils. The further conclusion can be drawn that the detrimental factor in ordinary soils is favoured by the water and organic matter present in sewage-sick soils. All our results are in complete agreement with the view that the detrimental factor consists in amoebae and other protozoa.

We have further studied the economic problem of applying our results to the treatment of sewage-sick land, particularly with the view of ascertaining whether partial sterilisation of the soil caused increased purification of the effluent, or whether any modifying factors come into play when the experiment is conducted in the open field.

A number of small land filters were made, 24 inches in diameter and 8 inches in depth when the soil had shrunk to its minimum volume. These were filled with partially "rested" soil some of which was heated rather strongly, some treated with 0.07 per cent. of toluene or of carbon disulphide and some left untreated. The filters were set up in the field and received sewage daily at the same time as it was run on to the land. The effluents were sampled periodically and analysed, with results set out in Table VII.

In the early days of the experiment the effluents from the treated soils were not as good as those from the untreated soil, but after about a fortnight they are seen to be distinctly better. The samples collected on October 20 may be taken as typical of the effluent during the autumn, and show satisfactory purification, especially when the shallowness of the filter is considered. The November sample was taken not long after the filters had frozen. It ran through the soil very quickly in consequence of cracks produced by the frost, and hence was not well purified, but still the treated soils give the best effluents. The February samples were taken so long after frosts that all danger of cracks was past, but the purification effected by the untreated soil is

not so good as in the earlier samples, indicating that sewage sickness is setting in. The treated soils, on the other hand, show no signs of falling efficiency; they are fully as effective as in November, although not quite as good as in the autumn.

TABLE VII. *Composition of effluents from small filters which contained partially sterilised soils. Parts per 100,000.*

	Free and saline ammonia				
	Aug. 12 1910	Aug. 26	Oct. 20	Nov. 19	Feb. 18 1911
Crude sewage . . . . .	9.50	9.00	5.48	15.00	13.50
Effluent from untreated soil . . . . .	1.61	1.38	1.84	1.50	3.88
Soil treated with 0.07% carbon disulphide . . . . .	3.56	3.03	1.26	1.03	1.90
Soil treated with 0.07% toluene . . . . .	2.92	0.85	0.40	0.30	0.65
Burnt soil . . . . .	—	4.86	0.41	0.50	0.50

	Albuminoid ammonia				
	Aug. 12	Aug. 26	Oct. 20	Nov. 19	Feb. 18
Crude sewage . . . . .	1.40	0.72	1.28	1.85	1.25
Effluents from untreated soil . . . . .	0.59	0.28	0.22	0.31	0.35
Soil treated with 0.07% carbon disulphide . . . . .	0.92	0.31	0.16	0.27	0.20
Soil treated with 0.07% toluene . . . . .	0.62	0.31	0.16	0.22	0.21
Burnt soil . . . . .	—	0.57	0.17	0.20	0.06

	Oxygen absorbed				
	Aug. 12	Aug. 26	Oct. 20	Nov. 19	Feb. 18
Crude sewage . . . . .	5.6	2.32	—	10.14	7.84
Effluents from untreated soil . . . . .	3.3	1.78	1.08	2.44	3.70
Soil treated with carbon disulphide . . . . .	3.9	1.28	0.8	2.42	2.25
Soil treated with toluene . . . . .	3.0	1.38	0.78	2.30	1.23
Burnt soil . . . . .	—	2.75	1.6	1.36	0.75

A set of filters was made up corresponding exactly with those just dealt with, but receiving in addition lime at the rate of two tons per acre. The result was a rather strongly alkaline effluent much less pure than from the unlimed soils whenever, as not infrequently happened, the alkalinity rose to 9 or 10 c.c. of decinormal alkali per

100 c.c. of effluents. As the alkalinity of the effluent seemed to be the determining factor in the purification, the results need not be discussed here.

In all cases nitrates were present but no estimate of their amount was obtained.

A series of small plots was laid out, one being pared and burnt to a depth of three inches, another treated with toluene (commercial "toluole") applied at the rate of 350 lbs. per acre by means of a Vermorel injector, and a third treated with carbon disulphide at the rate of 350 lbs. per acre. The plots were then sown with turnips. From the outset the plot treated with toluene showed the best and most even lot of plants, the tap root only and not the bulbs being affected by finger-and-toe. The plot treated with carbon disulphide came next, followed by the pared and burned plot, and finally the untreated, the plants on the last two being irregular and much attacked by finger-and-toe. The crops, however, were all good, but those on the treated plots were the best, the weights of total produce being, in thousand pounds per acre:—

Plot treatment ...	Untreated	Pared and burned	Treated with Carbon disulphide	Treated with toluene
Total produce .....	26.4 } 30.2 }	31.8	32.6	36.8

Larger plots of one-tenth of an acre were set out on the College sewage farm at Kingston and drains were laid 18 inches deep—the greatest depth practicable, although insufficient for thorough purification—so that the effluents from each could be tapped. The soil is much heavier than at Kegworth and the sewage differs in character in that it contains a relatively large amount of effluent from the dairy and cheese-making department. The land slopes somewhat, but it was possible to get two pairs of comparable plots, one of which was treated on May 24 with commercial "toluole" at the rate of four hundredweights per acre, the injections being about three inches apart and six inches deep. General inspection on a number of occasions showed that the effluent from the toluole treated plot was the better in each case both in colour and in smell, and the same fact is shown by the analyses of the effluents. In Table VIII it is seen that the treatment effects a marked reduction in the amount of albuminoid ammonia present in the effluent and also somewhat reduces, except in one instance, the high amounts of free and saline ammonia.

TABLE VIII. *Partial analyses of the effluents from the College sewage farm, Kingston. Parts per 100,000.*

		Free and saline ammonia			Albuminoid ammonia		
		July 1, 1911	The same effluent kept till Aug. 1	Nov. 11, 1911	July 1, 1911	The same effluent kept till Aug. 1	Nov. 11, 1911
Pair A	Untreated .....	6·83	9·48	6·07	2·50	1·39	3·55
	Treated with toluole...	6·06	7·95	7·65	1·53	1·11	1·30
Pair B	Untreated .....	8·71	11·6	7·4	1·95	1·18	2·22
	Treated with toluole .	6·00	7·11	4·5	1·25	·77	1·75
Crude sewage .....		6·90	8·78		7·52	9·90	

<sup>1</sup> Satisfactory samples could not be drawn during August and September because the land was laid up to be cleaned from weeds and became so dry (the weather being very hot) that cracks formed and allowed some of the sewage to reach the drains direct. By the end of October the cracks had gone; the November sample represents an effluent that had really percolated through the soil.

These filter and plot experiments have not the same quantitative significance as the laboratory experiments because they are much less under control, but they show that no complicating factors come in on the large scale to change very much the character of the results. No essential difference need therefore be anticipated between the results of the laboratory experiments and of the field trials, and it seems probable that the method will find useful application in sewage farming once the mechanical difficulties of partially sterilising soil on the large scale are overcome. Mr Purvis has pointed out to us some of the economic advantages, such as the possibility of reducing the amount of land necessary for a given population, but into these questions we need not now enter. Our present conclusion is that partial sterilisation may be expected to give useful results wherever the rate of decomposition is limited by the number of bacteria. It removes one of the depressing agents and brings about an increase in bacterial numbers and consequently in the rate of decomposition. Of course if decomposition is limited by some factor other than bacterial numbers partial sterilisation will not necessarily effect any improvement.

*Conclusions.*

1. Two distinct sets of causes can be traced at work in sewage-sick soils: physical causes that lead to retarded percolation, and a factor detrimental to bacteria.

2. The factor detrimental to bacteria is in every respect similar to that shown by Russell and Hutchinson to exist in ordinary soils. It is put out of action by the same antiseptics and by heat; it is not bacterial nor is it any bacterial product; it is not carried by an aqueous extract of the soil. On the other hand it is transmitted to partially sterilised soils by inoculation with untreated soil. It is not put out of action by aeration or by lining.

3. Its effects are, however, much more pronounced in sewage-sick soil than in ordinary soil. While the bacteria in the untreated sick soil only rarely rise to 40 millions per gram they may rise to as many as 400 millions per gram in the partially sterilised soils. The high amounts of moisture and organic matter in the sewage-sick soil appear to be especially favourable to the harmful factor.

4. Sewage sickness is thus regarded, in part, as an abnormal development of the harmful factor always present in ordinary soils.

5. As in the case of ordinary soils, all the properties of the harmful factor indicate that it is biological and consists in organisms larger than bacteria. Examination of the untreated soil showed the presence of numbers of amoebae and other protozoa, some of which could be separated out in an active form by centrifuging. None were present in the partially sterilised soil. All the evidence at present available points to these as constituting the harmful factor.

6. After the harmful factor is killed by partial sterilisation the bacteria multiply rapidly and rise to high numbers, effecting more decomposition of the added sewage so that a purer effluent is obtained. No complicating factors appear to be introduced when the method is tried on the large scale, and there can be little doubt that once the practical difficulties of partially sterilising large quantities of soil are overcome it will find useful application in sewage farm practice wherever the rate of decomposition is limited by the numbers of bacteria.

# PROBABLE ERROR IN PIG FEEDING TRIALS.

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IN a recent number of this *Journal*<sup>1</sup>, T. B. Wood and F. J. M. Stratton have discussed the probable error of field and feeding experiments, but in the latter case their investigations included only cattle and sheep.

The following work was undertaken as a necessary preliminary to a series of pig feeding experiments which are in contemplation. It is published at once in the hope that the results obtained may be of use to others who may be engaged in planning similar feeding trials.

Among the many records of pig feeding trials, access to which we have been able to obtain, only two series are of any use for the calculation of probable error. In all the other records where any considerable number of pigs were under experiment, their individual weights were not recorded.

In a series of experiments described in the tenth annual report (1911) of the West of Scotland Agricultural College, several batches of 8 pigs were fed for 10 to 11 weeks, each on the same diet. The individual weights at the beginning and end of the experimental period are recorded. From these weights, the probable error on one animal, found by the usual least square method, works out for the different batches of 8 pigs at from 4 to 12 per cent. of the live weight increase. The pigs were of both sexes and of various sizes.

Probable errors have also been worked out from the weights of individual pigs recorded in the Report of the Wisconsin Experimental Station for 1895. Two batches of 10 pigs and three batches of 6 pigs were employed and the time of feeding varied from 9 to 15 weeks. No mention is made of sex and the pigs differed considerably in weight. The probable error on one animal works out at from 6 to 12 per cent. of the live weight increase.

As the results available for examination were so few, it was determined to carry out trials for the express purpose of finding the probable error. For this purpose, eighteen pigs were taken and housed in sets of three in six pens. They were fed on an *ad libitum* ration of sharps mixed into a slop with water. Barley meal was introduced in the ninth week and gradually increased in proportion to the sharps. It was

<sup>1</sup> *J. Agric. Sci.*, Vol. iv. Part 3.

arranged that the animals should always feed to repletion, and the troughs were taken out of the pens half an hour after each feeding time. During the first six weeks of the experiment the food was given three times a day, but, later, two meals a day were given. Records were kept of the amount of food consumed in each pen so that, if any trio of pigs failed to clear up their food, less was allowed for the following meal. Water was provided for drinking, but was seldom touched. Once a week, a pound of small coal was given to each pen of pigs.

Thirteen of the pigs were put under experiment on July 31st, 1911, but the other five were not obtained till August 7th, so that the experiment, as far as it concerned the whole eighteen animals, began on the latter date. All the pigs were of the large white breed, and the thirteen which started on July 31st were practically uniform in age and weight, while the other five did not differ greatly from these. All the pigs were castrated males and, at the beginning of the experiment, were on an average ten weeks old.

Weighings were made on July 31st, August 7th, August 26th, and afterwards at intervals of four weeks, the last weighing being on Nov. 21st.

Two pigs died during the experiment from causes which had nothing to do with the conditions of the experiment.

The following table shows the weights recorded.

*Weights of pigs in lbs.*

No. of pig	July 31st	Aug. 7th	Aug. 26th	Sept. 25th	Oct. 23rd	Nov. 21st
1	35	40½	52	74	102	—
2	33	37½	42	60	93	125
3	40	44½	54	78	103	142
4	44	47½	59	79	97	128
5	43	45	58	83	104	139
6	37	41½	51	75	100	133
7	35	37½	42½	59	89	119
8	34	37½	43½	59	83	112
9	40	44½	53	—	—	—
10	41	46	58	82	103	132
11	35	42½	45½	67	85	109
12	36	43½	52½	75	108	140
13	38	44½	54	77	106	129
14	—	31½	41	63	88	120
15	—	26½	33	47	72	104
16	—	28½	37	57	89	124
17	—	29½	35½	53	74	97
18	—	30½	38½	55	77	105



From these figures the following results were obtained by the least square method :

(1) For the whole number of pigs for the complete period the probable error of one animal was 8 per cent. of the average live weight increase.

(2) For the whole number of pigs, the probable error of one animal in the intervals between the weighings was as follows :

(a)	18	pigs	for	3	weeks	21·6	per	cent.	of	average	live	weight	increase.
(b)	17	"		4	"	11	"		"		"		"
(c)	17	"		4	"	13·4	"		"		"		"
(d)	16	"		4	"	9·6	"		"		"		"

The error, therefore, while greater than that for the whole period, tends to diminish as the pigs get older.

(3) For the eleven pigs which survived out of the original thirteen the probable error of one animal is 7·0 per cent. of the average increase.

(4) In the same way as in (2) we get the probable error of one animal as follows :

(a)	13	pigs	for	4	weeks	15·7	per	cent.	of	average	live	weight	increase.
(b)	12	"		"		11·2	"		"		"		"
(c)	12	"		"		13·8	"		"		"		"
(d)	11	"		"		8·0	"		"		"		"

Here, as in (2), the error diminishes as the pigs get older.

It will be seen that the probable errors calculated in (3) and (4) are little less than those in (1) and (2), showing that accuracy is not greatly increased by uniformity in weight among the animals under experiment.

Uniformity of breed, however, appears to be very important, for in a second experiment on nine animals, some of which were large whites, and some middle whites, the rate of increase of the two breeds differed so greatly that the experiment was discontinued.

From the results of these experiments and those obtained from the Scotch and American figures, it appears that the probable error of one animal in a pig feeding experiment is in the region of 10 per cent. of the average live weight increase.

Now, in a comparative feeding experiment, we aim at taking such a number of animals that the differences may be with certainty attributed to the effect of the two diets and not to normal variation.

It is shown, in the paper on probable error, quoted above, that a difference of 3·8 times the probable error ensures that differences due

to normal variation are practically ruled out of the question. The probable error of an average of  $n$  animals is obtained by dividing the probable error of an individual by  $\sqrt{n}$ . We can therefore construct a table showing the relation between the percentage live weight increase and the number of animals required to ensure that the differences are not due to normal variation.

Percentage difference in live weight increase to be expected under the conditions of the experiment	Number of animals required in each lot. (Fractions counted as the next highest whole number)
50	1
40	1
30	2
20	4
10	15
5	54

### CONCLUSIONS.

(1) The probable error of one result calculated from a four week period is large but is diminished as the animals get older and more accustomed to the diet.

(2) This error is much reduced by taking a longer period and 12 weeks may be suggested as the shortest period consistent with accuracy.

(3) The probable error of one animal in a mixed lot of approximately the same age and weight is very little more than that in a lot more closely approximating in these respects.

(4) A table has been prepared to show the number of animals which ought to be taken to show up varying differences with precision. For example, fifteen animals must be taken in each lot where the two methods of feeding are expected to show a 10 per cent. difference.

# THE ESTIMATION OF POTASSIUM, ESPECIALLY IN FERTILISERS, SOIL EXTRACTS AND PLANT ASHES.

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IT is still no uncommon occurrence when the same sample of a potash fertiliser is submitted to different analysts to find the results returned differing by very large amounts. In one case recently, that came within the writer's experience, the results of two of the best known London analysts differed by nearly 3% in the case of a sample of 90% sulphate of potash. The present paper contains an account of an investigation of the sources of error likely to affect the results in the methods now in use. The conclusion is drawn that the perchloric acid method which in Germany has almost superseded the platinum chloride method (Precht, *7th Intl. Congress, App. Chem.*, 1909, Section I, p. 146) is far superior to the older process not only on the ground of cost, but as being freer from error, simpler and less likely to give differences in the hands of different analysts. This method has been applied to the analysis of soil extracts and plant ashes and the conditions are described, by observing which it is possible to obtain very exact results free from the errors attendant on the use of platinum chloride.

## *Platinum Chloride Method.*

Considerable differences exist in practice as to the concentration of the alcohol used for taking up the syrupy mixture of sodium and potassium platinichlorides in order to separate the former, which is the more soluble. Many chemists adopt 80% alcohol as recommended by Fresenius (*Zeit. Anal. Chem.*, 1877, **16**, 63), but others follow Precht (*Zeit. Anal. Chem.*, 1879, 509) in considering that absolute or "high-percentage" ("hochprozentig") alcohol is better. This is the practice of the Stassfurt chemists: Tietjens in his article on "Potash Salts" in Lunge's *Chem. Techn. Untersuchungs-methoden* (English edition, **1**, 520) considers the fact that potassium platinichloride is less soluble in absolute alcohol (1 in 40,000) than in 80% alcohol (1 in 25,000) to be an advantage, and that the more correct results obtained by many chemists using 80% alcohol are probably due to a "compensation of

errors." In an important paper by Morozewicz published in 1906 (*Bull. Acad. Cracovie*, 1906, 796) this question has been specially investigated. Morozewicz maintains that if absolute alcohol is used, not only are high results usually obtained with mixtures of sodium and potassium chloride, but that three or four times the quantity of platinum chloride is necessary to keep the sodium in solution as platinichloride as in the case when 80% alcohol is employed. The following experiments confirm these statements and show the absolute necessity, when the proportion of sodium chloride is considerable, of using 80% alcohol if anything like accurate results are to be obtained.

In the majority of the following experiments the potassium platinichloride precipitate was collected on a filter paper, previously dried in a weighing bottle at 100°, as in the Stassfurt method (Lunge, *Technical Methods*, I, 521). A solution of potassium chloride was used containing 1.0 grm. pure KCl per 100 c.c. 10 c.c. = 0.10 grm. KCl.

A. *Potassium Chloride alone.* Taken 0.10 grm. KCl, 2.5 c.c. of a solution of  $\text{PtCl}_4$  containing 6.8 grms. Pt per 100 c.c. (= 0.17 grm. Pt; theoretically required = 0.13 grm. for  $\text{K}_2\text{PtCl}_6$ ).

TABLE I.

Expt. No.	Conditions	$\text{K}_2\text{O}$ taken	$\text{K}_2\text{PtCl}_6$ weighed	$\text{K}_2\text{O}$ found*	$\text{K}_2\text{O}$ found $\text{K}_2\text{O}$ taken	Remarks
1	10 c.c. KCl solution = 0.10 gram KCl, 2.5 c.c. Pt solution	0.0632	0.3310	0.0639	101.1	$\text{K}_2\text{PtCl}_6$ taken up with 95% alcohol; total vol. of alcohol used for washings = 100 c.c.
1 <sup>i</sup>	Same ppt. $\text{K}_2\text{PtCl}_6$ washed with 50 c.c. AmCl solution (AOAC) then with 50 c.c. 95% alcohol	0.0632	0.3345	0.0646	102.2	
1 <sup>ii</sup>	Ppt. from 1 <sup>i</sup> washed with 50 c.c. 80% alcohol	0.0632	0.3272	0.0632	100.0	
1 <sup>iii</sup>	1 <sup>ii</sup> washed with another 50 c.c. 80% alcohol	0.0632	0.3265	0.06305	99.8	
2	Duplicate of 1	0.0632	0.3310	0.0639	101.1	
2 <sup>i</sup>	Duplicate of 1 <sup>i</sup>	0.0632	0.3375	0.0652	103.1	
2 <sup>ii</sup>	Duplicate of 1 <sup>ii</sup>	0.0632	0.3270	0.06315	99.9	
2 <sup>iii</sup>	Duplicate of 1 <sup>iii</sup>	0.0632	0.3240	0.0626	99.0	

\* Using the Stassfurt factor 0.3056 for converting  $\text{K}_2\text{PtCl}_6$  to  $2\text{KCl}$ ; this assumes an atomic weight for Pt = 197.2, not the atomic weight 194.8 which is now generally accepted, but is based on the fact that the precipitated platinichloride dried at 100° is not pure  $\text{K}_2\text{PtCl}_6$ , but contains water of crystallisation.

† The ammonium chloride solution saturated with  $\text{K}_2\text{PtCl}_6$  was prepared according to the official American (AOAC) method, Bulletin No. 107, U.S. Dept. of Agriculture.

It will be seen that by using 95% alcohol even with pure potassium chloride slightly high results are obtained; if the precipitate is washed with ammonium chloride and then with 95% alcohol, the results are still higher (owing no doubt to precipitation of  $\text{AmCl}$  by the concentrated alcohol), but on washing with 50 c.c. of 80% alcohol, the results become practically the theoretical. Further washing with another 50 c.c. of 80% alcohol lowers the results owing to the  $\text{K}_2\text{PtCl}_6$  beginning to dissolve in the dilute alcohol.

*B. Potassium Chloride and Sodium Chloride.*

Taken 0.10 gm.  $\text{KCl}$ , 0.10 gm.  $\text{NaCl}$ , 5 c.c. Platinum solution  
(= 0.34 gm. Pt. Required for  $\text{KCl}$  and  $\text{NaCl}$  = 0.30 gm. Pt).

0.10 gm.  $\text{KCl}$  taken = 0.0632  $\text{K}_2\text{O}$ .

TABLE II.

Expt. No.	Conditions	$\text{K}_2\text{PtCl}_6$ weighed	$\text{K}_2\text{O}$ found	$\frac{\text{K}_2\text{O found}}{\text{K}_2\text{O taken}}$
3	Using 105 c.c. 95% alcohol in all for taking up and washing	0.4010	0.0774	122.5
3 <sup>i</sup>	Ppt. in 3 washed with 50 c.c. 80% alcohol	0.3250	0.0628	99.4
3 <sup>ii</sup>	Ppt. from 3 washed with further 50 c.c. 80% alc.	0.3237	0.0625	98.9
3 <sup>iii</sup>	3 <sup>ii</sup> again washed with 50 c.c. 80% alcohol	0.3200	0.0618	97.8
3 <sup>iv</sup>	3 <sup>iii</sup> again washed with 50 c.c. 80% alcohol	0.3160	0.0610	96.6
4	Using 107 c.c. 95% alc. for taking up and washing	0.3905	0.0754	119.3
4 <sup>i</sup>	Ppt. in 4 washed with 50 c.c. 80% alcohol	0.3250	0.0634	100.4
4 <sup>ii</sup>	4 <sup>i</sup> washed with additional 50 c.c. 80% alcohol	0.3265	0.0631	99.8
4 <sup>iii</sup>	4 <sup>ii</sup> washed again with 50 c.c. 80% alcohol	0.3230	0.0624	98.7

It is seen that, even with such small quantities as 0.10 gm.  $\text{KCl}$  and 0.10 gm.  $\text{NaCl}$ , by using 105 c.c. of 95% alcohol in all, for taking up and washing, very high results are obtained; the 95% alcohol was naturally added in small quantities at a time, as in the Stassfurt method, 20 c.c. being used to take up the syrupy platinichlorides. On once washing the precipitate on the filter paper with 50 c.c. of 80% alcohol practically correct results are obtained (0.0628 and 0.0634 gm.  $\text{KCl}$  instead of the 0.0632 gm. taken), but subsequent washing steadily causes loss of weight, owing to the solubility of the potassium platinichloride, and the results become lower and lower with successive washings.

That the high results obtained when 95% alcohol is used are due to co-precipitation of sodium chloride with the potassium platinichloride is shown by the fact that on washing the first precipitates 3 and 4 with 80% alcohol and evaporating the washings so obtained in a glass

dish, white transparent cubes of sodium chloride are observable under the microscope. Morozewicz has attributed this separation of sodium chloride to the decomposition by absolute alcohol of sodium platinumchloride in the sense  $\text{Na}_2\text{PtCl}_6 \rightarrow 2\text{NaCl} + \text{PtCl}_4$ ; and shows that when absolute alcohol is used, three times as much platinum chloride is necessary to keep all the sodium chloride in solution in the form of sodium platinumchloride as in the case when 80% alcohol is employed. In the latter case, however, a slight deficiency of platinum chloride below that required to form  $\text{Na}_2\text{PtCl}_6$  is not prejudicial to the results.

When using the platinum method, especially in estimating small quantities of potassium as in soil extracts, one of the greatest difficulties is to know precisely when the washing is sufficiently complete to remove the last traces of sodium platinumchloride; if washing is too prolonged, serious loss of *potassium* platinumchloride may occur—a loss, which when the actual amount of potassium platinumchloride weighed is small, may cause considerable error. Experiments were made to ascertain the amount of potassium platinumchloride actually passing into solution on washing the pure salt on a filter paper with successive 50 c.c. portions of 80% alcohol; the following results were obtained:

1st wash,	loss=	0.0015
2nd	„	„ =0.0020
3rd	„	„ =0.0025
4th	„	„ =0.0023
5th	„	„ =0.0022

Average 0.0021

From Precht's determination of the solubility of  $\text{K}_2\text{PtCl}_6$  in 80% alcohol, if the alcohol passed away saturated, the loss for every 50 c.c. of alcohol would be 0.0020 gm., which agrees closely with the figure obtained above.

This loss of 2 mgrms. for every 50 c.c. of washing alcohol is a serious matter when only small quantities of platinumchloride have to be weighed. The uncertainty of the amount of washing required, which is a difficulty in the platinum method<sup>1</sup>, is entirely avoided in the perchlorate method, where the loss of perchlorate is practically *nil* when the washing is carried out at first with 95% alcohol containing 0.2% of perchloric acid, the perchloric acid being finally removed by washing with a very small quantity of pure 95% alcohol until the washings no longer show any acidity to litmus paper (see p. 57).

<sup>1</sup> An obvious method of avoiding this difficulty would of course be to use in washing 80% alcohol which has been previously saturated with pure potassium platinumchloride.

In practice, some analysts evaporate the aqueous solution of the mixed platinichlorides nearly to dryness, then moisten with a small quantity of water and take up with 90 to 95% alcohol, subsequently washing with the stronger alcohol; the moistening with water tends of course to bring the concentration of the alcohol in the first washing nearer the 80% value and thus to render the results more correct than by using absolute alcohol throughout.

One of the greatest difficulties encountered in using the platinum method is met with when analysing sulphates, in which case the whole of the  $-\text{SO}_4$  has to be *very exactly* precipitated with barium chloride, as, for example, in the Stassfurt method. If there be a slight excess of barium chloride, or, on the other hand, of sulphuric acid, the platinum method gives erroneous results. The perchlorate method, on the contrary, gives perfectly accurate results even when a relatively large amount of barium chloride is present, or a considerable proportion of the potash exists as sulphate. In fact, as will be shown, when the perchloric acid is in large excess the potassium in potassium sulphate itself can be estimated exactly without any treatment whatever to convert the sulphate into chloride.

*To sum up:* the platinum method is liable

- (1) To give *high* results, unless 80% alcohol is used.
- (2) To give *low* results when 80% alcohol is used, owing to the relatively high solubility of  $\text{K}_2\text{PtCl}_6$ .
- (3) To necessitate great care when sulphates are present; high results may easily be obtained unless the precipitation of the sulphate by barium chloride is very exactly carried out.

In view of the many other real advantages possessed by the perchlorate method and the undoubted fact (as will be demonstrated later) that it gives accurate results without any very special precautions having to be taken, it is contended that the platinum method should be abandoned on account of its uncertainty, cost and special difficulties of manipulation.

#### *Perchlorate Method.*

The procedure adopted was as follows: when iron, aluminium or other salts are present, as in ordinary acid soil extracts, ashes, etc., the solution of chlorides is evaporated to dryness in a porcelain dish and ignited for about  $\frac{1}{4}$  hour at a dull red heat so as to throw out iron, etc., as oxides, as in Neubauer's simplified method (*Landw. Vers. St.*, 1905, 63, 141). The ignition should be continued so long that, on dissolving,

a colourless solution, free from iron, is obtained. [When sulphates are present in large amount, after the liquid has evaporated to dryness, 5 to 10 c.c. of saturated barium hydroxide solution is added to precipitate  $\text{SO}_4$ . The evaporation and ignition are then completed as usual.] The soluble alkali salts are then extracted with boiling water as completely as possible, breaking up the iron oxide residue with a glass rod during the extraction. The aqueous extract is filtered into a glass evaporating dish ( $3\frac{1}{4}$ " diam.), care being taken to extract all the alkali salts from the residue; this is usually complete with a volume of hot water sufficient to fill the  $3\frac{1}{4}$ " dish, but if any doubt exist, another 50 c.c. of boiling water can be used. The aqueous extract is now evaporated *nearly* to dryness after adding 2.5 c.c. of perchloric acid solution, sp. gr. 1.125<sup>1</sup> (20%); the evaporation must be carried to the point of vigorous evolution of heavy white fumes of perchloric acid. A sand bath not too strongly heated is the most suitable means of effecting evaporation<sup>2</sup>. The soluble perchlorates are now taken up by stirring with 20 c.c. of 95–96% alcohol, and after settling, the clear solution poured off through a 9 cm. filter paper, which has been dried to constant weight at 100° in a stoppered weighing bottle ( $1\frac{1}{2}$ " diam.  $\times$  2"). 10 c.c. of 95% alcohol is now added containing 0.2% of perchloric acid<sup>3</sup>, and the insoluble potassium perchlorate transferred as completely as possible by means of it to the weighed filter paper. In washing the last traces of precipitate into the filter paper another 20 or 30 c.c. of the alcohol containing 0.2% perchloric acid is used, and finally the perchloric acid itself is washed out of the filter as completely as possible by using a minimum of 95% alcohol. For this purpose the washings are tested, until quite free from acidity, with sensitive litmus paper. Care must be taken that the top edge of the filter paper is washed well. The freedom of the filter paper from perchloric acid is shown by its not blackening during the subsequent drying in the oven at 100°. The use of a glass dish for the evaporation of the solution in the early part of the process greatly simplifies the complete removal of the last traces of the perchlorate precipitate to the filter paper, as these last traces are then plainly visible. In washing,

<sup>1</sup> This quantity is sufficient in most cases, but if the quantity of  $\text{KCl} + \text{NaCl}$  exceeds 0.2 grm., 5 c.c. of perchloric acid or more should be used. It is necessary only to take about  $1\frac{1}{2}$  times the quantity of  $\text{HClO}_4$  theoretically necessary to decompose all the salts present.

<sup>2</sup> If the liquid evaporates to dryness no harm is done so long as there is no loss by spirting. It is only necessary to take up with a drop or two of the perchloric acid solution.

<sup>3</sup> Made up by adding 5 c.c. 20% perchloric acid to 500 c.c. 95% alcohol.



a total quantity of 120—150 c.c. of 95 % alcohol can be safely used without causing any perceptible loss of potassium perchlorate, although so much is not usually necessary unless much NaCl is present. After washing, the filter paper and precipitate are dried in a steam oven for about 20 mins. whilst still in the funnel, the filter paper plus precipitate is then transferred to its weighing bottle and the drying completed until the weight is constant. 1 mgrm.  $\text{KClO}_4 = 0.3401$  mgrm.  $\text{K}_2\text{O}$ .

A Gooch crucible or Soxhlet tube can also be employed for collecting the potassium perchlorate, but if this is used care must be taken that the asbestos layer is sufficiently thick to prevent the very finely divided potassium perchlorate from passing through. With a layer  $\frac{1}{8}$ " thick perfectly accurate results can be obtained. In rapid working, when a large number of analyses have to be made, the Gooch crucible is preferable to a filter paper.

### *Experiments.*

A. *Very small quantities pure Potassium chloride and sulphate mixed—no sodium.*

TABLE III.

Taken 0.0400 grm.  $\text{KCl}$  + 0.0100 grm.  $\text{K}_2\text{SO}_4 = 0.0307$  grm.  $\text{K}_2\text{O}$ .

With 0.050 grm. Fe present as ferric chloride.

Using 2.5 c.c. 20 % perchloric acid solution.

No.	Conditions	$\text{KClO}_4$ weighed	$\text{K}_2\text{O}$ found	$\frac{\text{K}_2\text{O found}}{\text{K}_2\text{O taken}}$
1	No barium chloride present	0.0920	0.0312	101.6
2	" " "	0.0897	0.0305	99.4
3	" " "	0.0910	0.0305	100.6
4	" " "	0.0900	0.0306	99.7
5	" " "	0.0905	0.0307	100.0
Average.....			0.0307	100.0

These results show that even when part of the potash is present as sulphate and no barium chloride is used to convert the sulphate into chloride, accurate results are obtained.

The following results were obtained by adding an excess of barium chloride to the solution whilst evaporating to remove the iron so as to convert all the potassium sulphate into chloride; the barium sulphate

formed was left with the insoluble ferric oxide on extracting, whilst the excess of barium passed into the potassium chloride solution. The above results are sufficient to show that such treatment is not actually necessary, but those which follow indicate that if such treatment is adopted in presence of a large proportion of sulphates or sulphuric acid, the precipitation need not be carried out very exactly, but a considerable excess of barium chloride can be present in the solution without interfering in the least. Such an excess would be, of course, utterly prejudicial to an analysis carried out by the platinum method.

TABLE IV.

Taken 0.0400 grm. KCl + 0.0100 grm.  $K_2SO_4$  + 0.05 Fe in the form of ferric chloride.

Using 2.5 c.c. 20% perchloric acid.  $K_2O$  taken = 0.0307 grm.

No.	Conditions	$KClO_4$ weighed	$K_2O$ found	$\frac{K_2O \text{ found}}{K_2O \text{ taken}}$	Remarks
				%	
1	2 c.c. of 1.27% solution* $BaCl_2$ , $2H_2O$ = 0.0182 $K_2SO_4$	0.0875	0.0298	97.1	BaCl <sub>2</sub> used nearly double the theoretical quantity to convert all the $K_2SO_4$ into KCl
2	" " "	0.0937	0.0318	103.6	
3	3 c.c. $BaCl_2$ solution = 0.0273 grm. $K_2SO_4$	0.0911	0.0310	100.9	BaCl <sub>2</sub> = nearly three times theoretical quantity
4	" " "	0.0889	0.0303	98.7	

\* 1 c.c. = 5.0 mg.  $SO_4$ .

The following results were obtained using 0.10 grm. pure potassium sulphate (in the absence of iron), evaporating down direct with 2.5 c.c. of 20% perchloric acid and collecting the perchlorate as usual. They show that under the conditions given the conversion of the sulphate into chloride by barium chloride is quite unnecessary.

TABLE V.

0.100 grm.  $K_2SO_4$  = 0.0541  $K_2O$ .

2.5 c.c. perchloric solution.

No.	Conditions	$KClO_4$ weighed	$K_2O$ found	$\frac{K_2O \text{ found}}{K_2O \text{ taken}}$
				%
1	0.10 grm. $K_2SO_4$ , no $BaCl_2$	0.1575	0.0536	99.1
2	" " "	0.1575	0.0536	99.1

*Estimation of Potassium***B. Potassium chloride + excess of sodium chloride.**

TABLE VI.

Taken 0.10 grm. KCl + 0.20 grm. NaCl = 0.0632 K<sub>2</sub>O.

No.	Conditions	KClO <sub>4</sub> weighed	K <sub>2</sub> O found	$\frac{\text{K}_2\text{O found}}{\text{K}_2\text{O taken}}$	Remarks
1	5 c.c. 20% HClO <sub>4</sub> solution	0.1870	0.0636	100.6	The solution of mixed chlorides was evaporated down direct with the perchloric acid solution and treated as usual
2	5 c.c. " " "	0.1855	0.0631	99.9	
3	2.5 c.c. " " "	0.1855	0.0631	99.9	
4	2.5 c.c. " " "	0.1860	0.0632	100.0	
Average...			0.06325	100.1	

The results are thus quite accurate in presence of twice as much sodium chloride as potassium chloride; 2.5 c.c. of perchloric solution is quite sufficient under the conditions given to transform all the sodium into soluble sodium perchlorate.

**C. Potassium chloride + excess of sodium phosphate.**Taken 0.10 grm. KCl + 0.20 grm. Na<sub>2</sub>HPO<sub>4</sub>, 12 H<sub>2</sub>O. Used 2.5 c.c. perchloric solution.0.10 grm. KCl = 0.0632 K<sub>2</sub>O.

TABLE VII.

No.	Conditions	KClO <sub>4</sub> weighed	K <sub>2</sub> O found	$\frac{\text{K}_2\text{O found}}{\text{K}_2\text{O taken}}$
1	2.5 c.c. 20% perchloric	0.1880	0.0639	101.2
2	" " "	0.1840	0.0626	99.0
3	" " "	0.1865	0.0634	100.3
Average.....			0.0633	100.2

In these experiments the solution was directly evaporated with the perchloric acid. It is seen that the presence of a large proportion of sodium phosphate does not in the least interfere with the accuracy of the results.

**D. Potassium chloride + calcium chloride.**

Taken 0.10 grm. KCl + 0.10 grm. CaCO<sub>3</sub> dissolved in dilute hydrochloric acid. The mixture was evaporated direct with 2.5 c.c. of 20% perchloric acid.

0.10 grm. KCl = 0.0632 grm. K<sub>2</sub>O.

TABLE VIII.

No.	KClO <sub>4</sub> weighed	K <sub>2</sub> O found	K <sub>2</sub> O found K <sub>2</sub> O taken
1	0.1850	0.0629	% 99.6
2	0.1875	0.0637	100.8
Average.....		0.0633	100.2

*E. Potassium chloride + magnesium sulphate.*

It was found that when magnesium sulphate is present in large proportion the results obtained are quite inaccurate; with 0.10 gm. MgSO<sub>4</sub>, 2 H<sub>2</sub>O present to 0.10 gm. KCl the results are 5 to 10 % high, and when 0.20 gm. magnesium sulphate is present the results are about 40 % high. In these cases the mixed salts were evaporated directly with the perchloric acid; evaporating the original solution to dryness and igniting, as when iron is present, and then using the aqueous extract for treatment with perchloric acid does not greatly mend matters (Experiments 5 and 6). But by adding 1 gm. of barium hydroxide to the original solution, evaporating, igniting, and then treating the filtered aqueous extract with perchloric acid in the usual way, exact results are obtained (Experiments 7 and 8). Experiments 9 and 10 show that when a solution containing ferric chloride, sodium phosphate

TABLE IX.

Using 2.5 c.c. 20% perchloric acid. 0.10 gm. KCl = 0.0632 K<sub>2</sub>O.

No.	Conditions	KClO <sub>4</sub> weighed	K <sub>2</sub> O found	K <sub>2</sub> O found K <sub>2</sub> O taken	Remarks
1	0.10 gm. KCl	0.2505	0.0852	% 134.8	Directly evaporated with the perchloric solution
2	+ 0.20 gm. MgSO <sub>4</sub> , 7H <sub>2</sub> O	0.2735	0.0930	147.2	
3	0.10 " KCl "	0.2045	0.0695	110.0	Directly evaporated with the perchloric solution
4	+ 0.10 MgSO <sub>4</sub> , 7H <sub>2</sub> O	0.1980	0.0673	106.5	
5	0.10 " KCl "	0.2345	0.0797	128.2	Original solution evaporated, ignited, and extract treated with perchloric acid
6	+ 0.20 MgSO <sub>4</sub> , 7H <sub>2</sub> O	0.2315	0.0787	124.6	
7	0.10 " KCl + 0.20 MgSO <sub>4</sub>	0.1870	0.0635	100.5	Evaporated with 1 gm. barium hydroxide, ignited, extracted, and treated as usual
8	+ 1.0 Ba(OH) <sub>2</sub>	0.1885	0.0641	101.4	
9	0.10 KCl + 0.10 MgSO <sub>4</sub> , 7H <sub>2</sub> O	0.1860	0.0632	100.0	Evaporated to dryness, ignited, and extract treated as usual. No Ba(OH) <sub>2</sub> used
10	+ 0.10 Na <sub>2</sub> HPO <sub>4</sub> , 12H <sub>2</sub> O				
	+ 0.05 Fe as chloride				
	" "	0.1845	0.0628	99.4	

and magnesium sulphate is evaporated to dryness, ignited and the extract treated with perchloric acid in the usual way, exact results are obtained without any prior treatment with barium hydroxide; but in treating solutions containing much sulphate it is always safest to add a little baryta. Considerable excess of baryta is without prejudice to the results.

That a large excess of magnesium, when present as *chloride*, is not prejudicial to the results, is shown by the following figures. In practice, this fact is of importance in the analysis of such materials as carnallite and kainite; in such cases, it is only necessary to convert the sulphates into chlorides by treatment with a slight excess of barium chloride, it being unnecessary to remove the magnesium, even when present in large quantity.

TABLE X.

0.10 gram. KCl + 10 c.c. of a solution of magnesium chloride prepared from pure magnesium oxide and containing in the 10 c.c. 0.282 gram.  $\text{MgCl}_2, 6\text{H}_2\text{O}$  (equivalent to 0.342 gram.  $\text{MgSO}_4, 7\text{H}_2\text{O}$ ).  
0.10 gram. KCl = 0.0632  $\text{K}_2\text{O}$ .

No.	Conditions	$\text{KClO}_4$ weighed	$\text{K}_2\text{O}$ found	$\text{K}_2\text{O}$ found $\text{K}_2\text{O}$ taken	Remarks
1	{ 0.10 gram. KCl + 0.282 $\text{MgCl}_2, 6\text{H}_2\text{O}$ }	0.1850	0.0629	99.6	{ Using 2.5 c.c. 20% perchloric acid solution }
2		0.1855	0.0631	99.8	
	" " "				" 5.0 c.c. " "

#### F. *Soil extracts.*

The following examples show comparisons of results given by the perchlorate method in the estimation of small amounts of potassium, with those obtained in the usual platinum chloride method as carried out at Rothamsted. The results are expressed in actual grms. of  $\text{K}_2\text{O}$ .

TABLE XI.

No.	$\text{K}_2\text{O}$ by $\text{KClO}_4$ method	$\text{K}_2\text{O}$ by $\text{K}_2\text{PtCl}_6$ washed with 80% alcohol	$\text{K}_2\text{O}$ by $\text{K}_2\text{PtCl}_6$ , treated with $\text{AmCl}$ solution and then with 80% alcohol (A.O.A.C. Bull. 107)
1	0.0250 gram.	0.0245 gram.	0.0225 gram.
2	0.0178	0.0178	0.0169
3	0.0104	0.0110	0.0097
4	0.0078	0.0084	0.0064
5	0.0076	0.0068	0.0047
6	0.0068	0.0084	0.0062
7	0.0231	0.0204	—

Some experiments were made to ascertain whether any loss of potash was to be feared owing to volatilisation during the ignition of the dry residue obtained by evaporating a soil extract; this ignition was carried out at a very dull red heat, over an ordinary  $\frac{1}{2}$ " Fletcher's Argand gas-burner, turned about two-thirds on. It was also possible that some potash would be retained by the ignited residue, as was actually found to be the case by Neubauer in the case of soil extracts containing a deficiency of calcium salts; to obviate this he recommended, in such cases, that a little calcium chloride be added (0.2 to 0.5 grm., in the form of pure calcium carbonate added to the acid extract). The experiments made to test these points were carried out with alluvial soils rich in clay and organic matter, which had been found to give difficulty in estimating potassium by the platinum method in use at the Rothamsted laboratory. As a type of this kind of soil, the Orgarswick soil, No. 236 (Romney Marshes), of the survey of Kent, Surrey and Sussex (Board of Agriculture, 1911), may be mentioned; it contains clay 20.3, loss on ignition 11.0%,  $\text{CaCO}_3$  0.42%,  $\text{K}_2\text{O}$  = 0.66%. It was found that such soils gave practically identical results with the perchloric acid method carried out as already described, with previous ignition, to those obtained in the survey; on adding, too, a known weight of potassium chloride to the soil extract and then proceeding as usual, practically 100 per cent. of the total potash present was accounted for in the analysis, as the following examples show:

TABLE XII.

No.	Conditions	$\text{K}_2\text{O}$ taken	$\text{K}_2\text{O}$ found	$\frac{\text{K}_2\text{O taken}}{\text{K}_2\text{O found}}$	Remarks
1	Soil 236 + 0.10 grm. KCl	0.0790	0.0782	99.0	5 c.c. 20% perchloric acid
2	Soil 52 + 0.10 grm. KCl	0.0842	0.0842	100.0	" " "

Many analyses, which need not be recorded here, have shown that, in dealing with practically all soil extracts, treatment with baryta (or barium chloride) is unnecessary, as the proportion of sulphates is generally very small. In analysing artificial plant-food solutions containing magnesium sulphate this treatment is, however, essential.

#### G. Ash Analysis.

The perchlorate method lends itself particularly well to the rapid and exact estimation of potassium in plant ashes, and in the ashes

obtained in evaporating crude liquors in the manufacture of organic acids (tartaric, citric, oxalic). The aqueous extract of the ash is filtered and, if necessary, evaporated to dryness with a small proportion of baryta, to remove sulphates; the residue is extracted with boiling water, filtered and the filtrate treated with 2.5 to 5 c.c. of perchloric acid in the usual manner. Very numerous analyses made by this method have convinced the writer of its rapidity and exactness.

*Loss of Potassium Perchlorate in washing. Use of Alcohol saturated with Potassium Perchlorate.*

All the foregoing analyses were carried out using 95—96 % alcohol containing 0.2 % of perchloric acid for the main portion of the washing, the last traces of perchloric acid being removed by means of the least possible quantity of pure 95 % alcohol (usually about 5 c.c.). The character of the results shows that by working in this way small error is incurred. But when the method was employed in other hands in the routine analyses of the laboratory, it was found that duplicate analyses frequently differed by several milligrams on the weight of the perchlorate. These differences were ultimately found to be due to too large a volume of alcohol being used in the final washing to remove perchloric acid. Experiment showed that the solubility of potassium perchlorate in pure 95 % alcohol is relatively high, each 50 c.c. dissolving from 0.0065 to 0.0085 gm. of the pure potassium perchlorate when passed through a Soxhlet tube containing the precipitate. The higher values were obtained when the alcohol was passed through slowly so as to give it a better opportunity of becoming saturated with the salt. The average value obtained in 12 experiments, carried out under conditions approximating to those generally used in washing, gave 0.0073 gm. per 50 c.c. of 95 % alcohol. On the other hand the amount dissolved under similar conditions by 95 % alcohol containing 0.2 % of perchloric acid is very much smaller, being about 0.0012 gm. per 50 c.c. of the alcohol (correction having been made for the 5 c.c. of ordinary alcohol used to wash out the perchloric acid).

It is clear, therefore, that in washing the perchlorate precipitate care must be taken to avoid dissolving traces of it whilst washing out the perchloric acid. To obviate this error, the simplest method is to use, instead of the 95 % alcohol containing 0.2 % perchloric acid, pure 95 % alcohol which has been previously saturated with potassium perchlorate at the temperature at which the actual washing

is carried out<sup>1</sup>. In this case care must be taken *to drain off, as completely as possible, the 20 c.c. of alcohol containing perchloric acid* (with which the precipitate was first taken up) before adding the alcohol saturated with perchlorate. If this precaution be not observed, traces of potassium perchlorate are thrown out of the saturated solution on mixing with the alcohol containing perchloric acid and the result is very slightly high. There is no necessity to wash finally with pure alcohol, as the error introduced by the trace of perchlorate present in the saturated alcohol is less than 0.0001 grm.

By using 95 % alcohol saturated with potassium perchlorate, the advantage is obtained that it is possible to wash with comparatively large volumes of alcohol if necessary (for example 100 to 200 c.c.) without any error being introduced owing to the precipitate dissolving; this is often necessary when other perchlorates, such as those of sodium and barium, are present in large amount. Its use, too, removes all uncertainty as to whether the washing has been efficiently carried out, as it is only necessary, after once weighing, to wash again with 50 c.c. of the alcohol saturated with perchlorate, dry and again weigh. The second weighing should not differ from the first by more than 0.0005 grm. Such treatment is desirable in estimating very small weights of potassium, and numerous analyses, which need not here be quoted, have shown that a high degree of accuracy is hereby attained.

#### SUMMARY.

It is shown that whereas the platinum chloride method of estimation is uncertain and liable to give varying results, the perchlorate method described is at once more simple in manipulation and more uniform and exact in its results. An improvement has been introduced which consists in washing the perchlorate precipitate with 95 % alcohol saturated with potassium perchlorate by means of which any error due to the solubility of the precipitate is obviated. This is of importance when dealing with small quantities of precipitate. The following other advantages may here be enumerated :

<sup>1</sup> As the solubility of the perchlorate in 95 % alcohol somewhat rapidly increases with rise of temperature, it is necessary to saturate the alcohol with perchlorate at approximately the temperature of working. This can easily be done by keeping a Winchester quart full of alcohol in contact with the powdered perchlorate and filtering off fresh quantities just before use. Experience has shown that alcohol saturated on a cold day will dissolve 1 to 2 mgrm. per 50 c.c. when the temperature rises from 75° to 80° F.



(1) *Economy.* In view of the very large excess of platinum required when sodium salts are present and the great cost of the metal at the present moment (£13 per oz.) this is a very real advantage. The troublesome working up of large quantities of platinum residues with the attendant risks of poisoning are avoided.

(2) The presence of barium, magnesium and calcium chlorides and sodium phosphate is without prejudice to the method, and these salts need not be removed. Potassium sulphate can be estimated direct, using a sufficient excess of perchloric acid, without conversion into chloride by means of barium chloride; if, in the analysis of commercial sulphate of potash, the sulphate is converted into chloride by the Stassfurt method, the *exact* precipitation of the sulphate is not imperative as in using the platinum method. There can be excess of either potassium sulphate or barium chloride.

(3) All uncertainty such as exists as to what value shall be taken for the atomic weight of platinum (see for example Precht, *Int. Congress, App. Chem.*, 1909, I. p. 145) is avoided. The calculation is made from

the simple molecular ratios  $\frac{\text{KClO}_4}{\text{K}_2\text{O}}$ ,  $\frac{\text{KClO}_4}{\text{KCl}}$ , etc.

## CIDER SICKNESS.

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THE present paper deals with the results of an investigation on a serious and common disorder of cider, generally known as cider sickness. The account here given is concerned with its characteristic features, its cause, and, as far as present knowledge permits, methods for its prevention. The disorder is due to the action of a bacterium. It is proposed to give a detailed account of the organism and its chemical activities in a supplementary paper.

Among the disorders to which cider is liable it is the most dreaded by cider makers in this country and causes the greatest loss, the extent of which has been estimated at several thousand pounds per annum. Its distribution is general in all the cider-producing districts, some of which, however, suffer much more severely than others. The heavy, sweet ciders typical of the best cider districts of Somerset are, for example, much more susceptible to attack than the lighter and brisker ciders of the Hereford type.

The disorder makes its appearance every year in ciders of the current season's making. It is generally during the month of May that the first symptoms of sickness may be observed in ciders most susceptible to the malady. From that time until the end of the summer outbreaks in more resistant ciders occur. The prevailing temperature has much to do with the exact time of the outbreak, a warm spring and early summer tending to cause an early appearance of the trouble, while a cool season checks its onset. The prevalence and intensity of the disorder vary considerably in different seasons, for which the character of the season's vintage as well as the summer temperature must be held responsible. There is little doubt that its occurrence is more widespread than is generally supposed. The features of the disorder are familiar to and quickly recognised by the more progressive makers, but in many cellars no distinction is drawn between it and a renewal of ordinary alcoholic fermentation due to yeast action.

Cider sickness is not confined to this country. It is apparently common in France and probably occurs also to some extent in other cider-producing countries. It is impossible to speak with much certainty on this point and about other matters concerned with manifestations of the disorder abroad, since the references are not sufficiently detailed to possess much diagnostic value. It seems probable that France and England are the only places which suffer severely, since the general type of fruit utilised for cider making in other countries is decidedly more acid in character than in the two countries named. As will be shown later, sharp ciders are not so liable to sickness as those of less acidity.

The malady is undoubtedly not one of recent introduction: but little satisfactory information can be gathered from the older writers upon cider on account of their failure to distinguish it from renewed normal alcoholic fermentation. No English authors appear to have given it special attention, nor have any detailed references to it been found in any of the modern German works on cider. The French literature on the subject is also scanty. Jacquemin refers to it in his *La Cidreterie moderne*, which is one of the most complete recent text-books on cider making, giving a very brief description of it under the name "Maladie de la pousse" and classing it with a disorder of the same name occurring in wines. The latter is familiar to wine makers and has been studied by Pasteur, Gautier, Duclaux, Laborde, Mazé and Pactollet, and other observers. A summary of their work is given by Semichon in his *Traité des Maladies des Vins*. From that account it is clear that, while there are many points of resemblance between cider sickness and the "maladies de la pousse et de la tourne" of wines, the disorders are not identical nor are they produced by the same organism.

We have not been successful in finding any fuller accounts of the disorder nor records of any investigations which have been made upon it: but the literature on cider is not easily accessible, and it is possible that some references may have escaped our notice.

The subject has attracted attention for some seasons past at the National Fruit and Cider Institute and a considerable amount of information with regard to it has been obtained from observations on ciders made there under practical conditions. Notes on the disorder were published in the *Annual Reports* of the Institute for 1906 and 1907. No serious work upon it was, however, attempted until the spring of 1911, when the organism which causes the disorder was successfully isolated in pure culture. Professor J. H. Priestley then

arranged for a part of the work to be conducted in his laboratory at Bristol: and our best thanks are due to him for placing it at our disposal and for his readiness to facilitate the work in every way.

### *The Characters of the Disorder.*

The following description of the characteristic features of the disorder is typical of most outbreaks; but considerable variations occur in individual cases. These will be dealt with in turn.

The earliest symptoms noticed in an attacked cider are a characteristic frothing of the liquor and a sudden and violent evolution of gas. Both bottled and draught cider are liable to attack. The first signs of trouble begin to appear generally early in May. By that time the ciders made during the preceding autumn and winter are fit for consumption, the normal alcoholic fermentation having practically ceased and the liquor being in a more or less quiescent and brilliant condition and almost free from deposit. The preliminary frothing which occurs at the onset of active sickness in bottled cider is slight but persistent, and may almost invariably be taken as a definite sign of the development of the disorder, if it appears in a cider from May onwards. If a bottle is opened at this stage, the contents are ejected with considerable force and so much frothing that it is difficult to collect any quantity of the liquid in a tumbler, the bulk being lost in the form of froth. In a few days sufficient pressure of gas is developed to cause the bottles to explode violently: and unless the corks are quickly released, the whole stock of the cider may be lost. Associated with this fermentative activity is a marked change in the aroma and flavour of the liquor, the original fruity character being lost or, more probably, overpowered by the development of a strong, peculiar and characteristic odour and taste. The sweetness is also reduced and in many cases eventually entirely disappears.

The next stage is the appearance of a haziness or slight turbidity in the hitherto clear liquid. Occasionally it is first observed almost simultaneously with the outbreak of fermentation. Generally it does not become strikingly noticeable until several days or, in extreme cases, weeks later. It increases gradually in intensity, until the liquor eventually attains a thick, milky condition. As the development of the turbidity proceeds, the colour of the cider apparently becomes paler. Part of this effect is doubtless due to the suspension of the creamy-brown particles of the substance causing the turbidity in the

liquid: but an actual loss of colour also undoubtedly occurs. As the turbidity increases, a thick deposit of an amorphous light brown substance is gradually thrown down.

After a lapse of time, in some cases extending to the following year, the liquor clears itself naturally, the whole of the material causing the turbidity being deposited as a crust on the sides and base of the receptacle. The characteristic "sick" flavour and aroma at the same time slowly disappear, and a dry cider of normal, though inferior, odour and taste is left. In this final condition it is drinkable, but its original market value is seriously reduced. Sometimes the turbidity is more persistent and the liquor does not become clear or fit to drink after protracted storage, traces of the "sick" flavour and odour being still retained.

Cider in cask is more liable to sicken than that in bottle. It is very noticeable that any disturbance, such as racking, at the critical season of the year tends to cause sickness to develop more quickly. In some instances it may escape entirely if undisturbed during hot weather, whereas if racked, sickness quickly follows.

Cider which has turned sick is generally apt, after the cessation of the violent fermentation, to acetify much more rapidly on exposure to air than sound cider similarly exposed.

While these features are typical of the disorder in its most complete form, several variations occur.

In some cases the whole series of changes already described takes place; but, instead of the fermentation proceeding until the sweetness is entirely dissipated, it stops prematurely, and the resulting cider then remains permanently more or less sweet.

Occasionally, especially in some bottled ciders, fermentation proceeds as usual, but the liquor never passes through the stage of turbidity. It remains clear, but a copious deposit is thrown down. Even the latter character may sometimes be lacking, the deposit being little more than that produced by the same cider remaining in a sound condition.

At times the fermentation itself shows considerable variation in intensity, often being very slight and in rare cases absent entirely, the only visible sign of sickness then being the clouding of the liquor, which is, however, accompanied by the "sick" flavour and aroma.

The development of this flavour and aroma is a feature common to all the instances referred to, and at present must be regarded as the sole constant character associated with the disorder. Instances of

sudden fermentations strongly resembling those occurring in sickness, except for the absence of the characteristic odour and taste, have been observed from time to time: but it has not yet been proved that they are in any way connected with sickness.

In accepting provisionally the occurrence of the characteristic flavour and aroma as the only constant features by which sickness may be diagnosed, attention may be called to certain abnormal instances of the occurrence of these characters. In 1906 among the samples of cider apples submitted to the National Fruit and Cider Institute for analysis was one sent from the Totnes district of Devon. While the juice was being expressed from some of the apples a strong odour of sickness was noticed in the laboratory, and on examining the freshly pressed juice it was found to possess the characteristic odour and flavour of sick cider. Each of the remaining apples of the sample was then tasted, and in every case the flavour of sickness was more or less strongly marked. The apples both outwardly and internally appeared perfectly sound and normal, and there was no trace of disease of any kind present beyond a few specks of the Apple Scab fungus, *Venturia inaequalis*, upon some of the apples. In the tissues of the fruit no bacteria or fungi could be found. The fruit from other trees of different varieties in the same orchard was quite normal in flavour. Specimens of fruit from the same tree have been examined in subsequent seasons, but in no case has the sick flavour been again discerned, the taste of the apples being normal in every respect. Until last autumn this was, as far as we know, the only case of the kind recorded. Certainly nothing similar had been met with among the several thousand samples of apples which were examined at the Institute during the years 1904-10. Last autumn, however, the same feature was observed in a number of different samples of apples analysed at the Institute. The apples affected were of several distinct varieties, quite different in character, and the fruit in many instances was grown in widely separate districts. The conclusion suggested by the facts was that the presence of the sick flavour was not due to the nature or variety of the apple nor to the soil or locality where the fruit was grown, but to abnormal chemical changes occurring during the later stages of the ripening. The flavour was generally most marked as the fruit approached the over-ripe condition. Presumably the abnormal character of the summer of 1911 was responsible for the common occurrence of the phenomenon. In no case could the presence of a diseased condition of the fruit, due either to fungi or bacteria, be associated with the development of the flavour. Probably,

therefore, the correct interpretation is that owing to certain abnormal physiological actions during the course of ripening the same chemical changes of certain constituents of the juice took place within the fruit, which occur in cider during the course of sickness as the result of bacterial action.

### *The Chemistry of Sickness.*

Consideration of the variations in the manifestation of sickness which have been observed shows that the leading changes occurring in cider during an attack of the disorder in its complete form are three-fold :

- a. The development of a characteristic aroma and flavour.
- b. The destruction of sugar accompanied by an evolution of gas.
- c. The production of a more or less dense turbidity and deposit.

a. The first change is probably due to the formation of small quantities of volatile ethers, aldehydes, and other organic substances possessing distinctive and powerful aromas. The effect is almost certainly composite and not due to the presence of a single substance. The chances of identification are small, since such bodies when produced during the course of fermentation are generally only formed in minute traces and cannot easily be isolated and identified. However, certain odoriferous substances have been successfully recognised. These include acetaldehyde, ethyl alcohol, and acetic acid. Higher alcohols, aldehydes, and fatty acids (including either butyric or valeric acid, and possibly both) are also present. The aroma and flavour characteristic of sick cider are, however, possibly due mainly to substances other than those already identified. The latter are formed in solutions of dextrose fermented with pure cultures of the sickness bacterium : but the odour and taste of such solutions, while perfectly characteristic, are entirely distinct from those of sick cider. The same remarks apply to other saccharine liquids fermented with the organism. Hence the aroma and flavour associated with sick cider are probably due largely to the formation of some unrecognised substance or substances from constituents present in apple juice and absent in the other liquids tested.

Since the chemistry of the fermentations caused by the bacterium is still under investigation and will be dealt with in detail in a subsequent paper, reference to that part of the subject in the following pages will be confined to the main features which have already been established.

b. During the course of fermentation of a cider in a state of active sickness there is a rapid fall in its specific gravity, accompanied by a marked loss of sweetness. Analysis has shown that this is owing to the destruction of the reducing sugars present in the liquor. The degree to which this destruction is carried differs considerably in individual instances, as will be gathered from the remarks already made with regard to the variability of the disorder. In extreme cases the diminution of the content of reducing sugars may be very slight, or practically complete. The consideration of the quantitative aspect may be conveniently deferred until the section dealing with the behaviour of the organism in sterilised media, since sick cider contains a variety of organisms in addition to the sickness bacterium, and their influence upon the chemical changes cannot be separated from that of the latter.

The reducing sugars present in cider of an age liable to be affected by the malady are almost entirely, and possibly exclusively, dextrose and laevulose. Their decomposition during sickness follows very closely along the lines of normal alcoholic fermentation by yeast, the main products being carbon dioxide and ethyl alcohol. (That the yeasts in the cider may play some part is admitted, but reference to the section dealing with the behaviour of the organism in sterilised sugar solutions will show the activity of the latter in this direction.)

The evolution of gas is rapid and considerable. At least 95 per cent. of the total amount is carbon dioxide, the remainder being mainly, if not entirely, hydrogen. Some recent experiments with nutrient sugar solutions have shown a destruction of sugar accompanied by the production of ethyl alcohol approximately equivalent to 50 per cent. of the weight of the decomposed sugar, without, however, the slightest sign of effervescence or the formation of more than a trace of free carbon dioxide. The significance of this departure from the normal behaviour has not at present been discovered.

The amount of ethyl alcohol produced during sickness may be considerable, and depends primarily upon the amount of sugar present in the cider at the moment of the development of the malady. It is not uncommon to find at a cider containing, for example, about 6 per cent. of reducing sugar and 3 per cent. of alcohol at the onset of sickness will within the course of a week or so lose practically the whole of its sugar and will double its content of alcohol. Indications point to the production of appreciable quantities of higher alcohols. Comparatively large amounts of glycerine are formed.



The most striking feature thus far observed distinguishing the decomposition of sugar during sickness from that by normal alcoholic yeast fermentation is the relatively large amount of aldehydes produced. The presence of acetaldehyde has been definitely recognised: and higher aldehydes in some quantity and formaldehyde are also formed. It is probable that these bodies play a prominent part in sickness phenomena in connection with the production of turbidity.

The decomposition of the sugar also gives rise to the formation of small quantities of fixed and volatile organic acids. Oxalic, acetic, and butyric acids have been found. Traces of lactic acid also may be formed, but tests for succinic acid have given negative results. The total acid production arising from the fermentation of the sugar rarely exceeds the equivalent of .2—3 per cent. malic acid. The actual changes in the acidity of the cider are somewhat complicated, since the organism apparently acts upon the malic acid present: and in moderately sharp ciders the total acidity may show a decrease during sickness in spite of the formation of some acids from the sugars.

c. The turbidity and deposit produced as the result of cider sickness are due partly to the increase in number of the organisms present in the cider. The increase in the bacterial content of the liquor is particularly noticeable. The main cause, however, is undoubtedly the formation of an insoluble substance or mixture of substances. Microscopical examination of a drop of the liquid reveals the presence of innumerable minute granules or resin-like droplets, frequently aggregated together in groups which both in size and form may easily be mistaken for colonies of bacteria of the coccus type. On heating the cider the turbidity to a large extent disappears owing to the solution of this material, which reappears again in its original form on cooling. Complete solution of the material is also effected by alcohol, if it has not been long precipitated. On long standing in the cider its solubility in alcohol appears to diminish considerably, possibly owing to changes in its constitution.

Its nature has not yet been fully investigated, but evidence already available points to its origin from the tannins, and possibly other related bodies, to which the colour of cider is largely due. For instance, its formation is generally most abundant in deeply coloured ciders containing a relatively large amount of tannin, and partial decolorisation of the liquor usually accompanies its appearance. From analogy with certain disorders of wines, such as *la pousse* and *la casse*, some such relationship might be anticipated. It is evidently produced from

constituents of cider not present in any of the ordinary nutrient solutions used for the cultivation of the organism, since no corresponding substance has been observed in the artificially prepared culture fluids.

Several facts suggest that it is formed as the result of the action of the aldehydes produced during sickness on the tannins or allied bodies in the cider. Pending definite proof, however, detailed discussion of the point may be postponed.

*The susceptibility of different types of Cider.*

Reference has already been made to the difference in the susceptibility of individual ciders to sickness. The records of all the ciders made at the National Fruit and Cider Institute during the past seven years serve to throw considerable light upon this aspect of the problem. These ciders have been made from selected varieties of vintage apples, which, instead of being mixed together prior to cider making according to the usual practice, have been dealt with separately. It has, therefore, been possible to ascertain the relations between the chemical composition of the variety, including variations due to soil and other factors, the type of cider produced from it, and the liability to sickness.

Cider apples may be divided into three classes according to the composition of their juices, viz.:

- a. *Sharp varieties*, the juices of which normally contain more than .45 per cent. of malic acid.
- b. *Sweet varieties*, with juices containing normally less than .45 per cent. of malic acid and less than .2 per cent. of tannin.
- c. *Bittersweet varieties*, yielding juices which contain normally less than .45 per cent. of malic acid and more than .2 per cent. of tannin.

As a general rule it is the ciders made from the two latter classes of apples which are susceptible to sickness, those made from the first class being resistant to the malady and usually escaping it entirely. The acidity of the cider is, therefore, evidently an important factor: and the records of individual examples prove that, *ceteris paribus*, the higher the acidity of the cider the more resistant it is to the disorder. The same rule holds good for ciders made from mixtures of fruit.

Since in some districts apples of low acidity predominate and in others sharp varieties are more abundant, it is evident that this is one reason why some localities suffer more than others. The kind of soil on which the fruit is grown also affects the acidity as well as the rate of fermentation of the juice, the importance of which is indicated in the following paragraph.

The amount of residual sugar in the mature cider is also important, sweet ciders suffering severely from sickness and well-fermented dry or nearly dry ciders rarely being noticeably affected. The quantity of sugar is dependent partly upon the method of treatment of the liquor during fermentation, but primarily upon the natural rate of fermentation of the juice. The degree of sweetness of a cider unsweetened by the addition of sugar may generally be taken as a very fair approximate guide to the natural rate of fermentation of the original juice, rapid-fermenting juices yielding under ordinary conditions dry ciders and slow-fermenting juices treated similarly giving sweet ciders.

Hence the greater susceptibility of sweet ciders may be due either to the presence of sugars in some quantity or to the slow rate of fermentation of the juices. Probably both factors are concerned. The presence of some sugar is necessary for the development of sickness to an appreciable extent; but the actual amount is in itself probably immaterial, although the results of the malady are most pronounced when the quantity is large. Since, however, the alcoholic content of the liquor is, roughly speaking, inversely proportional to that of sugar, and alcohol acts unfavourably on the growth of the organism, it follows that the amount of sugar present does in practice have some bearing upon the susceptibility to attack, independently of its relation to the rate of fermentation. The latter in turn, apart altogether from its bearing on sweetness, has much to do with predisposition to the disorder. In general, leaving out of account for the moment considerations of acidity influence, slow-fermenting juices yield susceptible ciders and rapid-fermenting types resistant ones. The explanation which suggests itself is that the yeasts are less well nourished in the former instances than in the latter owing to partial nitrogen starvation, and that consequently they are unable to dominate so thoroughly the flora of the cider, with the result that other organisms, including the sickness bacteria, have a better chance of development. (It may here be stated that the sickness organisms have in many cases been proved to be present in the freshly pressed juice. Probably this original infection rather than contamination at later stages is responsible for most outbreaks.) A vigorous primary fermentation by the yeasts should therefore be encouraged, although it is not invariably successful in warding off sickness, if the cider retains much unfermented sugar.

The liability of slow-fermenting and sweet ciders to sickness constitutes the most serious feature of the malady to practical cider makers, since they are generally of better quality than rapid-fermenting types and have a decidedly higher market value.

The storage temperature of the cider has also an influence upon the development of the malady. The warm season of the year is the signal for its appearance, and the hottest summers generally cause the greatest trouble. If a cider liable to sickness which has been stored at a cellar temperature of about 12° C. is placed at about 25° C., sickness usually appears in a few days, while a control sample left at the original temperature may escape entirely.

Tannin has been generally regarded as possibly the most important constituent of cider for the prevention of the development of its various disorders. In the case of sickness, however, there is clear evidence that its antiseptic properties are ineffective. The malady attacks liquors containing relatively high percentages of tannin as readily as those containing small amounts, if other conditions are equal.

Table A contains statistics of a few representative ciders of various types, illustrating the remarks made above as to the influence of the character of the cider upon its susceptibility to sickness. The specific gravity figures showing the rate of fermentation were taken from samples of the juices kept at a temperature of 27° C. The figures in large type indicate the appearance of sickness, the first in each instance denoting the point at which the disorder was first observed. The sudden drop in gravity between that and the previous record, succeeding a period of marked slackening in the rate of the primary fermentation, points clearly to the onset of the disorder independently of any other accompanying signs.

TABLE A.

Variety	Composition of fresh juice.			Course of fermentation, showing specific gravity at end of						
	Specific gravity	Malic acid %	Tannin %	2nd day	4th day	6th day	8th day	10th day	12th day	14th day
Ashton Foxwhelp ....	1·053	1·28	·376	1·048	1·031	1·021	1·013	1·008	—	—
Ashton Long Stem .	1·052	1·05	·286	1·045	1·036	1·032	1·028	1·027	1·027	1·027
Yellow Styre .....	1·059	·72	·184	1·043	1·024	1·015	1·007	—	—	—
Kingston Black, A ...	1·065	·65	·204	1·057	1·050	1·046	1·043	1·041	1·032	—
"      "      B ...	1·051	·60	·168	1·036	1·028	1·023	1·021	1·020	1·020	1·020
Lady's Finger .....	1·046	·58	·096	1·031	1·015	1·010	—	—	—	—
Horner .....	1·059	·36	·228	1·048	1·036	1·030	1·026	1·016	—	—
Morgan Sweet .....	1·046	·23	·160	1·041	1·007	1·001	—	—	—	—
Slack-ma-girdle.....	1·049	·19	·116	1·043	1·040	1·037	1·027	1·021	1·014	1·008
Royal Jersey .....	1·058	·20	·440	1·052	1·043	1·038	1·033	1·032	1·022	1·010
Twistbody Jersey .....	1·058	·22	·352	1·049	1·043	1·038	1·033	1·028	1·022	1·012
Improved Broadleaf...	1·045	·26	·446	1·042	1·034	1·017	—	—	—	—
Upright French.....	1·052	·32	·460	1·036	1·014	—	—	—	—	—

*Sickness in Perry.*

Perry, as might be anticipated from its close relationship as a beverage to cider, is also liable to attacks of the disorder. The above account of sickness as it affects ciders applies in almost every particular to perry also, even such characteristic features as the aroma and flavour produced by the malady being identical, or practically so. There is no occasion, therefore, to give a separate detailed account of it in relation to perry.

*The Organism of Sickness.*

Prior to this investigation it had been generally assumed that sickness was caused by the action of a specific organism or a group of organisms. In the early stages of this work definite proof was soon forthcoming, since sterilised ciders of a suitable type quickly showed all the symptoms of the disorder after infection with a few drops of actively sick cider; while, if the cider used for infection was sterilised before addition, no signs of sickness developed. A similar negative result occurred, if antiseptics were used in the place of sterilisation.

Biological examination of a sick cider has shown that its flora is very varied. The most abundant organisms are bacteria of several types. Many living yeast cells of diverse form are also present. The appearance of the latter, however, is not of a character to suggest that they are the cause of the malady; nor are they as numerous as would be expected if they alone were responsible for the trouble.

The attempt to isolate from sick cider an organism capable of reproducing the disorder was for a long time unsuccessful. None of the yeasts isolated possessed that property: and the only bacteria of note observed were acetic forms. Many series of fractional plate cultures were made from various sick ciders, both beer-wort, apple juice, and cider gelatine and agar media being used. Eventually last spring the sickness bacillus was successfully isolated from a set of beer-wort gelatine plates extra thinly sown from cider which had just begun to show the first symptoms of the disorder. The plate cultures in this case were kept at 22° C.; and it was not until the ninth day after infection that the colonies of the organism first became visible under a simple lens as minute dot-like growths. By that time the colonies of the other organisms present had developed so strongly that, if it had not been for the exceptionally thin sowing, those of the required bacterium would have been overgrown by the others and would have

escaped observation. On the 11th day isolation was possible. From the outset it looked probable that the organism was the one sought, since the number of its colonies far exceeded those of yeasts and acetic bacteria. Infections were taken from four of the colonies, two proving impure and the other two yielding pure cultures of a short rod-like bacterium, which showed itself in due course to be the same form in both cases. Further series of plate cultures were made from the two latter, the same type of colony developing in every instance, their purity thus being attested. From the latter series of cultures the pure stock cultures used in subsequent experiments were taken. Periodic repurification has been effected in the same way, a check thus being kept upon the validity of the results.

The earliest stock cultures were streaks on beer-wort gelatine. From them tubes of sterile beer-wort and cider were infected. After two or three days at 27° C. the former showed signs of active fermentation, but the latter remained apparently unaffected. The beer-wort fermentation did not at first, except for the production of a very frothy head, suggest sickness, the typical flavour and aroma being absent. The addition of a few drops of this actively fermenting wort to sterile cider was, however, sufficient to set up a vigorous fermentation in the latter within 48 hours at 27° C., a very frothy head being formed in this case also and a strong aroma and flavour of cider sickness developed. In course of time a thick milky turbidity was also formed. There was, therefore, no doubt then that the organism causing the malady had been obtained and that it had the power of producing all the phenomena of sickness in sterilised cider without the assistance of other organisms.

It is not proposed to describe here in detail the characters of the bacterium. Reference will be mainly restricted to those having a more or less direct bearing upon cider sickness.

In young cultures on most media, liquid and solid, the organism grows in the form of short, actively motile, rod-like cells, occurring singly or joined in pairs. The rods are generally about two-thirds as long as broad, the length ranging about  $2\mu$  and the breadth about  $1\mu$ . Their ends are slightly rounded. In old cultures the cells are frequently more elongated, and involution forms are common. The latter are very striking on some media, their length extending frequently to  $200\mu$  and their breadth being considerably reduced. The two ends in such cases swell up to globular structures  $25\mu$  or more in diameter. Forms of a dumb-bell shape are also common. No spore formation has yet been observed.

The organism is facultatively anaerobic. It grows on a variety of solid media, including most nutrient gelatines and agars, potato, carrot, and parsnip: but in no case is the amount of growth anything more than extremely limited, even at the optimum temperature and under either aerobic or anaerobic conditions. The rate of growth is also extremely slow. This limited and excessively slow growth on solid media is a very marked feature of the organism.

Growth is more abundant, both in solid and liquid media, in the presence of carbohydrates than in their absence.

The character of the growth on solid media is a creamy white, somewhat slimy mass, if the medium is on the moist side: if dry, the streak is non-spreading, rather darker in tint, and slightly cartilaginous in texture.

In liquid media containing carbohydrates growth is comparatively active, and is accompanied by more or less vigorous fermentation if dextrose or laevulose is present. The fermentation of laevulose appears to be much less active than that of dextrose. Saccharose, maltose, and lactose solutions never show signs of active fermentation, but occasionally the evolution of a few gas bubbles has been observed.

The gas given off during the fermentation of dextrose consists almost entirely of carbon dioxide. A small amount of hydrogen is also evolved, but quantities larger than 5 per cent. of the total gas production have not been noted. Ethyl alcohol is formed in some quantity, nearly 5 per cent. being produced at times from a 10 per cent. dextrose solution. A limited amount of acid is also formed. Acetic, oxalic, and butyric acid occur in small amounts. In a solution of commercial glucose alone (specific gravity 1·030) after eight days at 25° C. the specific gravity was reduced to 1·005, the alcohol formed amounted to 3·88 per cent., and the acidity was increased by the equivalent of ·07 per cent. malic acid.

A marked aroma, resembling somewhat that of decaying lemons in the earlier stage of fermentation and changing to a decidedly acid character in the later stages, accompanies the fermentation of dextrose: but on no occasion when sugar-containing liquids other than cider or perry have been used has any aroma resembling that typical of cider sickness been developed.

A temporary turbidity of the fermenting dextrose solutions occurs during the active stages of fermentation, but this quickly subsides at the cessation of fermentation. It is due apparently entirely to the cells of the organism in suspension and has no relation with that developed during cider sickness. In hopped beer-wort, on the other

hand, turbidity quickly appears and is very persistent. The presence of tannin in this case suggests that the production of the characteristic turbidity of sickness may be related to the occurrence of that substance.

The acidity of the medium has an important influence upon the extent of growth of the organism. It grows best in neutral or very slightly acid media, and its development is entirely inhibited in the presence of much acid. The most complete details with regard to the influence of acidity have been obtained from ciders in which the degree of acidity was varied by partial or complete neutralisation with caustic soda in some instances and calcium carbonate in others. The ciders used for this purpose were of the extremely acid type, the quantities of malic acid present ranging between 1 and 1.5 per cent. Where the acidity was reduced by neutralisation to points below the equivalent of .3 per cent. malic acid, growth of the organism was rapid and vigorous: between .3 per cent. and .5 per cent. it was moderate: above .5 per cent. conditions were obviously unfavourable and growth was irregular: above 1 per cent. it ceased. These results obtained by direct infection of sterilised cider correspond regularly with observations on ciders under normal conditions where the disorder has been allowed to develop naturally. There is some evidence to show that the organism may be capable of acclimatisation to the presence of considerable quantities of acid, and sickness has been produced in sterilised ciders containing as much as .9 per cent. malic acid. The resistance of the organism to the effect of acid and other unfavourable conditions depends very materially upon the vigour of the cells at the time of infection of the medium and upon factors influencing their nutrition.

Table B illustrates the effect of the organism on ciders in one of the series in which the acidity was varied by neutralisation with calcium carbonate. It will be noted that fermentation set in most rapidly where the acidity was lowest, and that the most acid sample was practically unaffected. The end-point in each of the other cases was approximately the same, fermentation ceasing suddenly for some unknown reason while the gravity was still relatively high. The total acidity, expressed in all cases in terms of malic acid, shows interesting variations, increasing in the ciders where it was low at the start and decreasing somewhat in the examples at the other end of the table. The results clearly indicate that the bacterium decomposes some of the malic acid originally present, the destruction being marked in the lower members of the series by a larger formation of other acids.

The organism grows best at comparatively high temperatures. Its



temperature limits for growth correspond closely with those ascertained for the development of sickness under practical conditions. The optimum temperature lies about 30° C. Between 20° C. and 35° C. growth is active. Above the latter point the rate slackens considerably, and no development has been observed above 40° C. Below 20° C. growth is also slower, and between 12° C. and 15° C. it is extremely slow. Below 12° C. there is hardly any appreciable development. The organism is killed by exposure to a temperature of 55°—60° C. for five minutes.

TABLE B.

<i>Cider.</i> Ashton Long Stem (specific gravity 1·036)	Specific gravity after infection			Acidity (expressed as malic acid % after infection		
	2nd day	5th day	8th day	2nd day	5th day	8th day
A. Acidity exactly neutralised with $\text{CaCO}_3$	1·019	1·012	1·012	·07	·13	·13
B. Acidity reduced to ·19% malic acid by partial neutralisation with $\text{CaCO}_3$	1·021	1·012	1·012	·27	·27	·28
C. Do. ·45% malic acid . . . . .	1·021	1·012	1·012	·45	·41	·43
D. Do. ·69% malic acid . . . . .	1·024	1·014	1·011	·61	·55	·53
E. Natural acidity, ·94% malic acid . . . .	1·036	1·036	1·036	·92	·92	·92

#### *Infection Experiments with Sterilised Ciders.*

Many experiments with sterilised ciders of different types in which sickness has been artificially induced by infection with pure cultures of the bacterium have been made, and in all cases the sickness has been in every respect similar to that which occurs naturally in the same ciders. The results of trials with ciders and perries of different types to determine their susceptibility to sickness and to ascertain the conditions which favour its development corroborate those which have already been stated as the outcome of observations on the disorder as it occurs under natural conditions. There is, therefore, no necessity to discuss in detail individual experiments nor to repeat the conclusions drawn therefrom.

The results with artificial infection do not, however, always coincide absolutely with those obtained under natural conditions. In some cases artificially infected ciders of comparatively high acidity have turned

sick, when the malady does not develop in the same ciders under natural conditions. This diversity in behaviour is accounted for partly by the fact that in artificial infections cells of the organism in most vigorous condition are used for inoculation, and partly because the heaviness of the infection largely determines the result. If such ciders are infected very lightly, sickness does not invariably follow, whereas a heavy infection causes sickness. Another possible factor is that under natural conditions the bacteria have to develop in a medium containing active organisms of other kinds, and they may be affected directly or indirectly by their presence. Where artificial infections have been made, on the other hand, the ciders used have previously been sterilised and thus the effect of other organisms is excluded.

#### *Methods for prevention of Sickness.*

This investigation of the disorder has suggested several lines of treatment for its prevention.

The occurrence of the bacterium is evidently widespread, and there is good evidence for believing that it is introduced into the cider direct from the fruit when the latter is milled and pressed. Whether it is a soil organism which reaches the fruit at the time of gathering, or whether like the cider yeasts it is normally found on the surface of the ripened fruit, is not at present known. It may be assumed with considerable probability that it is present in most ciders from the start, and that its subsequent history depends very largely upon the nature of the cider itself.

Measures for the prevention of the disorder, therefore, fall into two groups, those concerned with the elimination of the organism from the juice and those devoted to the production of a cider unfavourable to their development.

Under the former head the washing of the fruit prior to milling first merits attention. Washing with cold water has been repeatedly tried. While the results are better on the whole than those from unwashed fruit, they are nevertheless irregular and cannot be relied upon absolutely. Experiments on washing with hot water are now being tried, the results from which are not yet available. The work of Warcollier on the washing of the fruit with an antiseptic wash, such as very dilute formaldehyde, proves that juice practically free from any living organisms can be obtained: and possibly the method might be of service in this connection. While, however, it might be effective in

the hands of a careful worker with a knowledge of chemistry, it may be doubted if it would be judicious to recommend such procedure to cider makers in general.

Pasteurisation of the freshly pressed juice or mature cider also deserves consideration. Unfortunately under most conditions this form of treatment impairs the flavour of the cider.

To restrict infection as far as possible the sterilisation of all vessels and appliances which have been in contact with sick cider is to be recommended.

It must, however, be recognised that at present really satisfactory means for the elimination of the organisms from the juice or cider are lacking, and that the most efficient means of protection consist in the production of a liquor unfavourable for their development.

Since the presence of sugar is necessary for their growth, complete fermentation of the cider to dryness is effective: but it is not feasible in many cases on account of the extensive demand for sweet cider. It has, however, been found that, if juices of the slow-fermenting type are blended with others which ferment at a rapid rate, the product ferments moderately quickly and greater resistance to the disorder is secured than if the former are left unblended.

Another method favouring resistance is to raise the acidity of the cider to about 5—7 per cent. of malic acid by suitable blending. It is not practicable to increase the acidity of the beverage much beyond those limits, since the flavour becomes unpleasantly sharp.

It has also been found that the addition of a vigorous culture of yeast in the later stages of the fermentation of a susceptible cider has a beneficial effect, probably owing to the sickness bacteria suffering by competition with the active yeast rather than to the reduction of sugar during the increased fermentation, since there may still be a surplus of the latter after fermentation has been checked.

Fermentation and storage of the cider at as low a temperature as possible are obviously desirable.

The addition of an antiseptic such as salicylic acid is effective, but cannot be recommended on other grounds. Ciders which have been sulphured, are said to be strongly resistant, even after oxidation of practically the whole of the free sulphur dioxide.

In the case of bottled ciders it has been found that susceptible liquors if bottled very early in the season, *e.g.* in late January or February, frequently escape, whereas the same ciders bottled at the usual time in April almost invariably succumb. Perhaps the cause of

the difference in behaviour rests with the yeasts, which are much more active at the earlier period and charge the cider comparatively quickly with carbon dioxide then.

Although not invariably certain in their results, the measures recommended have been found in practical tests to give a very considerable degree of success: and there is fair promise that further experiments on similar lines under practical conditions may result in the elaboration of a sure method for holding the disorder more or less completely in check.

# INVESTIGATIONS ON "SICKNESS" IN SOIL.

## II. "SICKNESS" IN GLASSHOUSE SOILS.

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PREVIOUS investigations in this laboratory have shown that partial sterilisation of soil leads to increased productiveness. In attempting to apply this method on the large scale two courses were open. The more obvious was to seek for methods cheap enough for use in the field, and then to conduct a number of field trials to determine which was the best; this was almost certain to prove a tedious and expensive business and would not necessarily lead to a successful issue. The alternative plan, and the one we adopted, was to find classes of growers who could afford to use our present methods of partial sterilisation and who would be willing to do so. However restricted their number of crops might be we knew that the cost of the process must fall once it was applied in commercial growing, so that the range over which it was applicable would soon begin to widen; a further advantage was that from the outset we should be gaining experience of the working of partial sterilisation in practice. Fortunately we met with a large tomato and cucumber grower in the Waltham Cross district who put us in touch with the class of growers we wanted: in this way we came across the problem of sickness in glasshouse soils which forms the subject of the present communication.

It has long been known among growers that soils suffer a change under the intense system of culture prevalent in glasshouses, and gradually become unfitted for the continued growth of a crop. This deterioration—technically known as "sickness"—is perhaps best seen in commercial cucumber houses, where it may be so marked as to necessitate the soil being thrown away after a single season's use. It

also occurs in tomato houses, but it sets in more slowly, so that the soil may last for four or five seasons. Other instances are known, but we have confined ourselves to these two cases, which are typical of the rest, because we have at hand in the Lea Valley a large area under glass where cucumber and tomato sick soils occur in abundance.

The difference in the effective life of the soil in the two cases is probably not connected with any feature of the plant but with the conditions of growth. A commercial cucumber house in the Lea Valley is run at a very high pitch. The "borders" in which the plants grow are made up of alluvial pasture soil mixed with an equal weight<sup>1</sup> of straw manure and a liberal addition of bone meal. After the high temperature of the fermentation has subsided the soil is maintained by artificial heat at about 80° F. (26·7° C.) and is kept so moist that water can be squeezed out simply by the pressure of the hand. Each week during vigorous growth more manure is added, commonly cow manure water and artificials, while at frequent intervals dressings of lime or chalk and of new soil are given. Enormous crops are obtained, but at the end of the season—September or October—the soil ceases to be effective and even after lying uncropped till February, when the next season's borders are made up, it cannot be used again but has to be thrown out, its enormous manurial residues being sacrificed. The amount of the annual loss cannot be estimated, but it must be very great; at one nursery alone we saw over 5000 tons of this soil, which on analysis proved to be richer than farmyard manure (Table I). The new soil wanted for each season's work has to be purchased and carted, often from a considerable distance.

Tomato houses are run at a much lower pitch. Dung is put into the bottom spit during trenching, but little or no organic manure is added to the top spit in the first instance, fertilisers being used only when the fruit has set and has attained the size of peas. Care is taken to avoid excess of manure and water throughout, the temperature also is kept down, 65° F. being usually aimed at. It is commonly agreed that soil will not profitably carry tomatoes for more than three seasons, but growers who grow in the ground and not in pots cannot afford to throw out the old soil. Instead they trench the ground every second or third year, carefully burying the top spit and bringing up the bottom spit; of late years it has become customary to apply an antiseptic such as a dilute emulsion of carbolic acid or of some tar oil to the top soil before burying it; sometimes also antiseptic is applied to the bottom spit after it has been brought up. Under these conditions the soil

<sup>1</sup> Some growers use less, but this is a common mixture.

seems to last indefinitely and "sickness" never sets in; we know of one house where tomatoes have already been grown continuously and successfully for eleven years without any sign of deterioration; here the trenching has been done annually and the antiseptic treatment has been very thorough.

"Sickness" is commonly accompanied by insect and fungus pests, perhaps the most frequent being *Heterodera radicicola* which produces the swellings on the roots both of cucumbers and of tomatoes—the so-called "club."

A comparison of the conditions of cucumber and tomato houses with other cases that have come under our notice leads to the conclusion that "sickness" of soil is associated with high organic matter content, high water content, and relatively high temperature. Where all these conditions are simultaneously present, as in cucumber houses, "sickness" quickly sets in; where only two are present, as on soils heavily dosed with sewage, it takes a longer time (see p. 27); it is even slower to appear in the poorer, drier and cooler tomato soils, where it may reveal itself only by the disease organisms, and finally, going still further down the scale of intense culture, it is no longer heard of in arable soils except in the case of leguminous crops which may form a wholly different case. We can, in fact, trace a perfect gradation from the extreme case of the cucumber house to the ordinary arable field soil.

It has been shown by Russell and Hutchinson that ordinary field soils contain a biological factor detrimental to bacteria, the effect of which is to keep down the bacterial numbers and the rate of production of plant food. This result suggests a simple explanation of "sickness"; the conditions being favourable to high bacterial development also favour a corresponding but slower (p. 98) development of the destructive organisms, till finally, when after many disturbances the new equilibrium sets in, the bacterial efficiency of the soil has fallen too low to maintain the high rate of production called for in a commercial glasshouse. On this hypothesis "sickness" is simply an instance of the limitation of the bacterial population that goes on in every soil, and it should be amenable to treatment by partial sterilisation.

Experiments showed that this expectation was well founded. After partial sterilisation "sick" soils behaved in precisely the same manner as normal arable soils, but to a much more marked extent. The fauna of the soil was simplified, amoebae, ciliates and higher forms being killed, the bacterial numbers rose considerably, the rate of production of plant food increased and larger crops were obtained.

But this simple hypothesis is incomplete because it takes no account of the parasitic or disease organisms present in sick soils. The conditions of the glasshouse industry favour a transfer of organisms from one glasshouse to another, while the conditions of a cucumber house are often not unlike those of a moist chamber in an incubator, so that once an organism appears it may multiply very rapidly. Thus the soil in the cucumber borders becomes inhabited by a remarkably varied population: growths of myxomycetes and other low vegetable forms can be seen on the surface, while active amoebae, eelworms and other low animal forms can be got out by simple centrifuging. No particular disease organism occurs invariably so far as we know, but there is generally evidence that plants in sick soils have been attacked by some or other organisms: whether this is one of the primary causes of the sickness or whether it is only a secondary effect consequent on a weakened physiological condition of the plant we are not in a position to say. In a tomato house the living population is less complex, but it includes many disease organisms.

These disease organisms are an undoubted factor in soil "sickness," indeed we might have considered them the sole agents, as Kühn did long ago in beet sickness on the Continent<sup>1</sup>, and Bolley has recently done in the flax and corn-sick soils of Dakota<sup>2</sup>, had we restricted ourselves to vegetation experiments. But while the destruction of the disease organisms by partial sterilisation might be supposed to account for the increased growth of crop it does not account for the increased bacterial population and rate of production of plant food, unless we suppose that the bacteria in the "sick" soils have been suffering from some disease. There is nothing absurd in this idea, but it is difficult to reconcile with the fact that addition of bacteria from the "sick" soils to the partially sterilised soils leads to further increases, and not decreases, in bacterial population and in the rate of plant food production (p. 97). Only when "sick" soil itself is added to the partially sterilised soil (our destructive organisms not being contained in the water extract) does any reduction set in.

In the scattered and fragmentary literature dealing with "sickness" various other hypotheses have been put forward, two of which are of sufficient importance to merit attention. It has been supposed

<sup>1</sup> *Bied. Centr.* 1880, 859-865.

<sup>2</sup> H. L. Bolley, "Conservation of the purity of soils in cereal cropping," *Science*, 1910, **32**, 529-541; also *Bulletins of the Agricultural Experiment Station for North Dakota*, Nos. 50, 55 and 87.



- (1) that the "sick" soils gradually acquire some substance toxic to plants,
- (2) that they acquire a substance toxic to bacteria.

We have gone carefully into the question of soluble plant toxins and failed to find any evidence of their presence in these soils. Cucumbers grown in an aqueous extract of a "cucumber-sick" soil showed no signs whatever of being poisoned, although other experiments proved that they are very sensitive to traces of poison in the solution. They started at least as well as plants grown in extracts of partially sterilised soils from which the harmful factor has been put out of action, and only fell behind when the food supply was nearing exhaustion. Further, they kept a healthier colour than plants grown in a culture solution, and they did better than plants grown in water alone, although in the latter case root growth started more quickly (Figs. 3 and 4, Plates III, IV, V).

If plant growth were limited by a plant toxin we should expect to find unabsorbed nitrates and ammonia in the soil after the plant has finished growth. This, however, we failed to do: at the end of the experiment the untreated soil is depleted almost as completely as any of the others. We must thus attribute the sickness in the first instance to the diminished bacterial activity we find in the sick soils.

Two consequences follow from this diminished bacterial activity. The decomposition processes go on more slowly, so that relatively low quantities of the simple nutritive decomposition products are present whilst relatively high amounts of complex decomposition products occur. There is evidence that some of these latter may be directly harmful to the plant, although they are not sufficiently soluble to appear in the aqueous extracts. As soon as the bacterial activity increases the soil conditions become more suitable to the plant.

Our experiments thus lead to the conclusion that at least two factors are concerned in soil "sickness": a falling off in bacterial activity and an accumulation of plant parasites and disease organisms. The factor keeping down bacterial numbers possesses identical properties with that present in arable soils; we must provisionally regard it as being the same. No bacterio-toxin could be found; the experiments showed that the factor is biological and non-bacterial. All these harmful organisms are more readily killed than bacterial spores, and partial sterilisation has the effect of leaving a clear field for the bacteria and a healthy soil for the plant. The treatment is effective for some time, since it is known (p. 98) that the re-establishment of the organisms destructive to bacteria is a slow matter, and the plant makes such vigorous growth on

partially sterilised soils that it is less liable than before to take disease even if the organisms get in.

Partial sterilisation therefore appears to be the proper method of dealing with "sick" soils. It is not entirely new. Certain large growers have for some years been in the habit of steaming their old cucumber soil or treating it with carbolic acid to kill "club" and other disease organisms, while many tomato growers treat their soils annually with antiseptics for the same reason. But apart from these special purposes the method is not in use, and we have extended our experiments to ascertain the lines on which large scale trials should be made with the end to make the treatment a regular part of commercial greenhouse practice.

Of the methods of partial sterilisation investigated, exposure to a temperature of 96°–98° C. for two hours has proved the most effective because it not only kills destructive and parasitic organisms, including *Heterodera*, but also effects a certain amount of decomposition, thus lightening the subsequent work of the bacteria and bringing about certain secondary results, notably a great development of fibrous root. This treatment is practicable on the large scale<sup>1</sup>. A temperature of 55° C. maintained for 3 hours suffices to kill destructive and parasitic organisms and thus to remedy sickness, but it does not effect the secondary changes. Toluene and carbon disulphide, the antiseptics we have most studied, produce satisfactory improvement in the bacterial numbers and the rate of formation of plant food, but they do not kill all the *Heterodera* nor do they bring about the useful secondary changes. But for practical purposes an application of antiseptic would prove more convenient than heat if the purely mechanical difficulty of distribution were overcome. The antiseptic should be some cheap substance or mixture of substances that can (1) put out of action the factor injurious to bacteria, (2) kill *Heterodera* and the spores of parasitic disease fungi in the soil, (3) when its work is done disappear from the soil by volatilisation, oxidation or other decomposition, leaving no permanent bad effects behind, (4) if possible lead to the same fine root development as a temperature of 98° C. We find at least six substances or classes of substances that more or less satisfy the first three conditions:

- (1) formaldehyde;
- (2) the lighter hydrocarbons of the tar oils: benzene, toluene, and the higher homologues present in the so-called light solvent naphtha and heavy solvent naphtha;

<sup>1</sup> See Russell and Petherbridge, *Journ. Board of Agric.* 1912, xviii. 809–826.

- (3) the heavier hydrocarbons of the naphthalene fraction ;
- (4) the tar acids : phenol, cresylic acid, etc. ;
- (5) the tar bases : pyridene and the homologues ;
- (6) calcium sulphides.

Pl. II, fig. 1 and Pl. III, fig. 2 show some of the results obtained. Of these the lighter hydrocarbons are not convenient for large scale work because of the cost of transport (they being classed as "dangerous goods" by railway companies) and the difficulty of application. The others are more suitable ; they are carried at ordinary rates and are readily put on to the soil because they are or can be made miscible with water. Further investigation is necessary to discriminate between the various substances. None of them, however, causes the fine fibrous root development obtained on heated soil ; for practical purposes steaming therefore remains the best of all the methods we have tried.

### *Experimental.*

The "sick" soils were collected in lots of two cwts. or more from large nurseries in the Lea Valley, chiefly in the region between Enfield Lock and St Margarets, and examined by the three methods that have given us the most useful results in other fertility investigations, viz. analysis, determination of the rate of production of ammonia, and vegetation experiments. The analytical determinations call for no special comment, their object being to ascertain whether the soil presents any marked peculiarity or deviates to any notable extent from the normal.

The rate of production of ammonia is measured by the sum of the ammonia and nitrate present at a given time. For this purpose a quantity of the soil is mixed very uniformly and divided into a number of 800 gram lots, some of which are left untreated as controls, while others are treated in the various desired ways. The different lots are then moistened so that all shall contain the same amount of water, they are put into litre bottles stoppered with cotton wool plugs, and kept in a dark cupboard. At definite intervals bottles are taken out for the determination of nitrates and ammonia. In untreated soils the amount of ammonia is only small because its rate of formation under normal conditions is slower than its rate of conversion into nitrates<sup>1</sup>. The determination affords a measure of the decomposability of the nitrogenous organic matter of the soil and an index of the amount of nitrogen

<sup>1</sup> This *Journal*, 1910, 3, 233.

likely to become available for a crop grown under similar circumstances. The method is based on the assumption that the ammonia and nitrates are neither being assimilated by micro organisms nor suffering any other decomposition except conversion one into the other. Control experiments have always to be made to find out whether the assumption holds good. An independent series of experiments is therefore started in which the soil is mixed with known weights of ammonium salts and of nitrates and kept under the same conditions as in the main series, determinations being made from time to time to see whether the added salts are recovered entirely as ammonia and nitrate.

Recovery appears to be complete in ordinary arable soils and in glasshouse soils, but as the method is open to criticism pot experiments are always made as a control. Equal amounts of the variously treated soils are put up into pots, non-leguminous crops are grown, weighed and analysed. The weight of nitrogen in the crop is obviously the final measure of the amount of nitrogen the plant could extract from the soil unless growth was limited by some other factor, in which case some of the nitrates will be left behind unassimilated. After the crop is removed, determinations are therefore made of the residual nitrates in the soil. In normal cases they amount only to about five parts per million of soil.

### *The analysis of the soils.*

Fairly complete examinations have been made of two cucumber sick soils and three tomato sick soils, and less complete examinations of three other soils; the analytical results are shown in Table I. The cucumber sick soils are extraordinarily rich, especially in phosphates and potash, as might have been expected from their manuring. A considerable amount of calcium carbonate is also present and there is no sign of acidity; indeed, so far as our experience goes, the soil in the cucumber house even when described as sour is alkaline because of the ammonia that is evolved from the manure. "Sickness" therefore cannot be attributed to lack of lime or plant food or to the presence of acids in the soil.

The tomato soils are poorer, especially *MT* and *SB*, which have been cropped for five and seven years respectively; the richest of the series *RC* 1 had been a cucumber soil, but was steam sterilised and mixed with new turf and one-eighth its weight of straw manure and used for tomatoes. In these cases also there is no sign of acidity or deficiency of lime.

TABLE I. *Percentage composition of sick soils, air dried.*

	Cucumber sick soils			Tomato sick soils				
	<i>A</i> *	<i>B</i> *	<i>Orl.</i>	<i>RC 1</i>	<i>SC</i>	<i>MT</i>	<i>M</i>	<i>SB</i>
Moisture.....	7.3	5.1	3.1	2.3	1.2	1.8	.9	1.7
Loss on ignition .. .. .	18.7	19.9	16.9	8.7	7.9	6.0	8.0	6.0
Total nitrogen .. . . .	.75	.72	.63	.37	.33	.26	.32	.22
Nitrogen as nitrates...	.027	.021	.017	.016	—	.005	—	—
"    ammonia .....	.0015	.0015	.0015	.0005	—	.0004	—	—
Calcium carbonate .....	1.07	.92	1.93	.57	—	.97	—	.63
P <sub>2</sub> O <sub>5</sub> sol. in conc. HCl ...	.56	.73	.55	.39	.31	.39	—	—
P <sub>2</sub> O <sub>5</sub> sol. in 1% citric acid	.36	.47	.29	.21	.17	.23	—	—
K <sub>2</sub> O sol. in conc. HCl ....	.54	.50	.26	.45	.46	.44	—	—
K <sub>2</sub> O sol. in 1% citric acid	.12	.10	.18	.08	.06	.07	—	—

\* *A* and *B* were from nurseries not far apart, the soil being made up from the same pasture land ("turf"). In spite of the admixture of an equal weight of dung and the frequent dressings of manure the organic matter and nitrogen have not increased very much. The composition of the original "turf" was:—

Moisture	Loss on ignition	Nitrogen	CaCO <sub>3</sub>
6.26	13.49	.59	.15
P <sub>2</sub> O <sub>5</sub> (in HCl)	P <sub>2</sub> O <sub>5</sub> (in citric acid)	K <sub>2</sub> O (in HCl)	K <sub>2</sub> O (in citric acid)
.16	.016	.39	.08

The nitrogen forms about 4 per cent. of the organic matter instead of the 3 per cent. found in ordinary arable soils; in the pasture soil from which *A* and *B* were made up the value is 4.4.

*The effect of partial sterilisation on the rate of production of plant food.*

(a) *Cucumber sick soils.* Treatment of the soil with 0.5 per cent. of toluene causes an instantaneous production of ammonia, the amount of which rises from 15 to 25 parts per million of soil (Table II). Heat, especially to a temperature above 90° C., has a much more drastic effect, increasing the ammonia to 74 parts per million and causing so much decomposition of organic matter that the water extract of the soil, instead of being a golden yellow colour, becomes brown. There is, however, no evidence that the solubility of the phosphorus or potassium compounds in citric acid solution is increased by the treatment.

When the soil is moistened and stored ammonia is gradually formed and is converted into nitrates in the untreated soil, and the soil heated to 55°, but accumulates unchanged in the soil heated to 98°, till finally more than 300 parts per million are present, an extraordinary high

TABLE II. *Effect of heat and toluene on cucumber sick soils.*A. Soil *OxL*. Immediate effect.

	Untreated soil	Partially sterilised soil		
		Treated with toluene	Heated 2 hrs. at 98°	Heated 2 hrs. at 55°
N as ammonia, parts per million of dry soil .....	15	25	74	38
N as nitrate, parts per million of dry soil .....	274	272	267	277
P <sub>2</sub> O <sub>5</sub> sol. in 1% citric acid, per cent.	·29	·30	·28	—
K <sub>2</sub> O " " "	·18	·17	·17	—

## Subsequent effect, 45% moisture being present.

	N present as ammonia, per million of dry soil			N present as nitrate, per million of dry soil			Total, ammonia + nitrate, per million of dry soil		
	At start	After 15 days	After 43 days	At start	After 15 days	After 43 days	At start	After 15 days	After 43 days
Untreated soil	15	16	16	274	279	315	289	295	331
Soil heated to 98°	74	264	338	267	239	288	341	503	626
" " 55°	38	11	13	277	333	392	315	344	405

B. Soil *OxL*. 42·4% moisture present.

	N present as ammonia, per million of dry soil		N present as nitrate, per million of dry soil		Total, ammonia + nitrate, per million of dry soil	
	At start	After 43 days	At start	After 43 days	At start	After 43 days
Untreated soil	13	21	315	347	328	368
Soil treated with toluene	23	134	295	325	318	459
" " CS <sub>2</sub>	20	143	282	326	302	469
Soil heated to 98° for 2 hrs.	64	255	323	342	387	597

amount that we have seen equalled only in partially sterilised sewage sick soils.

Another experiment is recorded in the second part (B) of the Table. A slow decomposition has gone on in the untreated soil, the total nitrate and ammonia increasing by 40 parts per million,

equal to 12 per cent. of the amount initially present. A much more rapid decomposition occurs in the soils treated with toluene and carbon disulphide, and a marked accumulation of ammonia takes place; the total nitrate and ammonia now increase by 140 and 170 parts per million, these gains being equal to 44 and 55 per cent. of the respective initial quantities.

The soil heated to 98° shows a still greater rate of decomposition and ammonia accumulation, the total amount of nitrate and ammonia now rising to 600 parts per million, a gain equal to 54 per cent. of that originally present in the heated soil and 82 per cent. of that originally present in the untreated soil.

A simultaneous set of experiments showed that the accumulation of nitrate and ammonia in the partially sterilised soils is not the result of throwing out of action some agency present in the untreated soil that removes nitrates and ammonia. Some untreated soil was divided into three portions, two of which received small quantities of ammonium sulphate and sodium nitrate respectively; after 43 days the nitrate and ammonia were estimated; the results are given in Table III.

TABLE III. *Amounts of ammonia and nitrate recovered from soils receiving known weights of  $(\text{NH}_4)_2\text{SO}_4$  and  $\text{NaNO}_3$ . Parts per million of dry soil.*

	N present as ammonia		N present as nitrate		N present as ammonia + nitrate		Percentage of added N recovered
	At start	After 43 days	At start	After 43 days	At start	After 43 days	
Soil <i>OxL.</i>							
Untreated soil alone . . . . .	13	21	315	347	328	368	—
„ „ + (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> . . . . .	118	21	325	519	443	540	150
„ „ + NaNO <sub>3</sub> . . . . .	14	21	453	485	467	506	99
Soil <i>RC.</i>							
Untreated soil alone . . . . .	5	3	108	136	113	139	—
„ „ + (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> . . . . .	88	3	122	245	210	248	109
„ „ + NaNO <sub>3</sub> . . . . .	5	4	215	240	220	244	105

In all cases recovery is complete and we may conclude that the increased quantities of ammonia and nitrate in the partially sterilised soils are the results of greater bacterial activity there.

Although bacterial counts were not made in our experiments they were taken in parallel experiments made in conjunction with Dr Hutchinson, and they showed a marked increase in numbers in the tolued soil, but no increase in the untreated soil—indeed there was usually a fall. The following are typical results:

	Millions of bacteria per gram of soil			
	At start	After 13 days	After 25 days	After 70 days
Untreated soil .. .. .	65	41	22	23
Soil treated with toluene...	8	187	128	182

It is thus evident that the untreated soil contains a factor limiting the numbers and activity of the bacteria but put out of action by toluene, carbon disulphide or heat. This factor is not a soluble toxin, for it is not present in the water extract of the untreated soil, in fact addition of such a water extract increases the numbers of the bacteria<sup>1</sup>. Nor is the factor any group of bacteria, for on reintroducing the original flora into the partially sterilised soil<sup>2</sup> there is not a fall, but a rise in numbers. It is not a toxin produced by bacteria, since it shows no signs of setting up in partially sterilised soils kept free from infection, in spite of the high degree of bacterial activity. The data on which these conclusions are based have been ascertained in conjunction with Dr Hutchinson and are dealt with fully in another paper, for convenience typical results are given here showing the conditions in the soil "*OxL*" 15 days after treatment:

	Bacteria, millions per gram	N as ammonia	N as nitrate	N as ammonia + nitrate
Untreated soil .. . . .	82	17	343	360
Soil treated with toluene .. . . .	245	99	281	380
Soil treated with toluene + water extract of untreated soil.....	408	101	303	404
Soil treated with toluene + 0.5% untreated soil .. . . .	287	107	291	398

Finally the factor can be introduced by adding somewhat larger quantities of untreated soil, *e.g.* 5 per cent., when the bacterial numbers

<sup>1</sup> The water extract contains bacteria.

<sup>2</sup> Done by infecting with 0.5% of untreated soil.



go down, indeed the smaller inoculation (0.5 per cent.) sometimes suffices, but so far as our experiments have gone it is not introduced in any other way:

	Bacterial numbers, millions per gram		Ammonia + nitrate, per million of dry soil	
	After 15 days	After 70 days	After 15 days	After 70 days
Toluened soil + 0.5 % untreated soil	233	255	162	389
" " + 5 % " "	243	117	176	270

The factor is slow growing and takes some time to assert itself.

Thus a complete parallelism can be traced between these sick soils and the normal arable soils investigated by Russell and Hutchinson, and the conclusion seems irresistible that similar agents are at work in both cases keeping down the bacterial numbers, but their effect is more marked in these rich sick soils than in the poorer arable soils.

The increased bacterial activity of the partially sterilised soils is not wholly expended in the production of plant food. There is a considerable loss of nitrogen from the partially sterilised soils relative to the untreated soils: the percentages after three months were:

In the untreated soil	...	...	...	...	853
In the soil treated with toluene	...	...	...	...	837
" " CS <sub>2</sub>	...	...	...	...	818
" heated to 98°	...	...	...	...	800

Other experiments showed that the denitrifying organisms still survive in the tolued soils and effect an immediate reduction in the amount of nitrate directly the air supply is cut off by overwatering. It is therefore wrong to assume, as is sometimes done, that the beneficial effects of partial sterilisation arise from any suppression of bacterial decompositions causing loss of plant food; as a matter of fact all bacterial decompositions appear to be accelerated.

### *The search for soluble toxins.*

We have made a careful search in sick soils for soluble substances toxic to plants, but the results have been wholly negative. A water extract of the sick soil was made by stirring up the soil with ten parts of water, allowing to stand for about 16 hours, and then decanting off

the turbid liquid into the culture vessels. In the first series of experiments the liquid was filtered, but later on this operation was omitted lest the filter should cause any complication. Cucumbers were then grown in these extracts.

Pl. III, fig. 3*a* shows the effect on the plants observed after 5 days, when ordinary distilled water was used. In 1 (food solution) growth has started, but not very well, in 2 (sick soil extract) the plant is doing remarkably well, in 3 and 4 (extracts of partially sterilised sick soils) growth is only beginning. Ordinary distilled water, however, is somewhat toxic to plants. Another experiment was therefore made in which the whole of the water used for the food solution and the soil extract was distilled in a silver still and collected in glass, so as to get as pure a liquid as possible. At first the plants in the untreated soil extract were at least as good as those in the other extracts, but after a fortnight those in the tolued soil extract began to grow rather more than the others, finally the tolued soil and heated soil extracts gave somewhat larger crops than the untreated extract. The figures are given in Table IV and show that the maximum difference was only 14 per cent. The extracts have not all the same initial composition since both toluene and heat cause a certain liberation of ammonia from the soil: thus some of the extracts contained in parts per million:—

Extract made from	Cucumber sick soil "OrL."			Tomato sick soil "SB"		
	Free and saline ammonia	Albuminoid ammonia	Nitrate	Free and saline ammonia	Albuminoid ammonia	Nitrate
Untreated soil	0.92	3.2	17.8	0.45	1.84	10.6
Tolued soil...	1.82	2.8	15.6	0.65	1.80	11.7
Soil heated to 55°	3.38	3.6	—	0.43	1.49	10.6
" " 98°	3.54	4.5	—	0.98	1.95	11.1

These figures are reflected in the growth of the plant: while in the early stages the untreated soil extract proved at least as favourable as the others, it sometimes gave rather poorer results later on, when the supply of food was beginning to be exhausted.

A set of cultures was made in which tap water was used<sup>1</sup>. This proved a very good medium for plant growth, and plants started in

<sup>1</sup> Harpenden tap water is hard, containing a good deal of calcium bicarbonate in solution.

it rather more quickly than in the soil extracts. Again, however, the untreated extract was at least as good as the partially sterilised extracts. (Pl. IV, fig. 3*b*.)

Finally, more concentrated extracts were tried, one part of soil being taken to two of tap water. The results were of the same kind as before, the plants in the untreated soil extract starting fully as well as in the others, and falling off only when the food supply began to be an important factor.

That the cucumber plant is susceptible to traces of poison in culture solution is shown in another experiment with *OxL* soil (Pl. IV, fig. 3*c* and Table IV), where the food solution and extracts were made with ordinary distilled water which in our laboratory is prepared in a copper

TABLE IV. *Growth of cucumbers in aqueous extracts of untreated and of partially sterilised "cucumber sick" soils.*

	Soil extracts					
	Food solution	Sick soil	Partially sterilised soils			
			Treated with toluene	Heated to 55°	Heated to 100°	
i. Soil <i>A</i> . Silver distilled water used.						
Wt. of dry matter produced—Root	·0375	·0395	·0742	—	·0646	
Shoot	·287	·1868	·1845	—	·1878	
Total, grms. . . . .	·3245	·2263	·2587	—	·2524	
Length of root, cms. . .	21·6	49·4	63·1	—	59·9	
ii. Soil <i>OxL</i> . Tap water used (Pl. IV, fig. 3 <i>b</i> ).						
	Water alone					
Wt. of dry matter produced—Root.....	0·25	0·4	1·3	1·8	—	1·3
Shoot .....	0·9	1·9	4·7	4·0	—	4·2
Total, grms.	1·15	2·3	6·0	5·8	—	5·5
Wt. of green shoot ...	6·1	25·9	44·3	34·7	—	37·6
iii. Soil <i>OxL</i> . Ordinary distilled water used (Pl. IV, fig. 3 <i>c</i> ).						
Wt. of dry matter produced—Root	·0085	·027	·025	·042	·022	
Shoot	·110	·219	·163	·231	·183	
Total, grms. ....	·1185	·246	·188	·273	·205	
Length of root, cms. . . .	3·1	18·2	23·5	28·1	15·9	

still, and therefore contains minute traces of copper. Little or no growth occurred in the food solution although growth was normal in the soil extracts. The retardation sometimes noticed in the extracts of heated or tolued soils can in like manner be attributed to some toxin, possibly excess of ammonia, produced by the treatment. Our interest, however, is in the untreated soil extract; in no case could we find that this extract was less suitable to the early stages of growth of cucumbers than the extract of partially sterilised soils. We have therefore no reason to suppose the presence in the "sick" soils of a soluble plant toxin that is absent from the partially sterilised soils.

The following considerations militate against the view that the effect of partial sterilisation is to remove a toxin from the untreated soil:

(1) Cucumber seeds are very sensitive to unfavourable conditions but they germinate fully as well in sick soil as in partially sterilised soil.

(2) Young seedlings are also very sensitive but they make perfectly satisfactory growth in sick soil till the food supply becomes an important food factor.

(3) Washing a sick soil with much water does not improve it as a medium for the germination of seeds and the growth of seedlings.

#### *The partial sterilisation of tomato sick soils.*

The results of the experiments with tomato sick soils are of precisely the same kind as were yielded by cucumber sick soils. Table V shows that the immediate effect of partial sterilisation is to increase the amount of ammonia in the soil, but not to increase the solubility in 1 per cent. citric acid of the potash or the phosphoric acid. Subsequently there is a marked increase in the rate of production of plant food and in the bacterial numbers.

As in the case of the cucumber soils, addition of traces of untreated soil to partially sterilised soils led to still further increases in bacterial numbers and in the rate of decomposition. Thus the original bacterial flora is more potent both for multiplication and decomposition than the new flora developing after partial sterilisation: when the new and the old are placed under equally favourable conditions by growing both on partially sterilised soils, the old proves to be the more effective. We therefore cannot attribute the beneficial effects of partial sterilisation to any change in the type of the bacterial flora or to any supposed

TABLE V. *Effect of heat and toluene on tomato sick soils.*

Soil MT. Immediate effect.

	Untreated soil	Partially sterilised soils		
		Treated with toluene	Heated 2 hrs. at 98°	Heated 2 hrs. at 55°
N as ammonia, parts per million of dry soil .....	4	5	19	10
P <sub>2</sub> O <sub>5</sub> sol. in 1% citric acid, per cent.	·20	·20	·20	—
K <sub>2</sub> O " " "	·075	·070	·071	—

Subsequent effect, 14% of moisture being present.

	Ammonia, parts per million of soil			Nitrate, parts per million of soil			Ammonia + nitrate, parts per million of soil		
	At start	After 32 days	After 114 days	At start	After 32 days	After 114 days	At start	After 32 days	After 114 days
Untreated soil .....	4	8	8·5	46	54	57	50	62	65·5
Soil treated with toluene	5	44	35	44	56	58	49	100	93
Soil heated to 98° for 2 hours.....	19	53	78	59	67	71	78	119	152
Soil heated to 50° for 2 hours.....	10	5	6	51	62	72	61	67	78

Soil RC. 23% of moisture present.

	Ammonia		Nitrate		Ammonia + nitrate		Bacterial numbers, millions per gram	
	At start	After 46 days	At start	After 46 days	At start	After 46 days	At start	After 46 days
Untreated soil .....	6	4	83	104	89	108	48·5	66
Soil treated with toluene	6	24	81	99	87	123	4·4	120
" " CS <sub>2</sub> ...	6	44	82	83	88	127	1·7	110

stimulus. It is necessary to insist on this point because some bacteriologists still assume that such a change or a stimulus takes place, although they offer no evidence in support of the assumption.

These bacteriological studies were made in conjunction with Dr Hutchinson and the details will be found in another paper written jointly with him. The following typical results may be given here,

showing the numbers of bacteria in millions per gram of the variously treated soils :—

	At start	After 21 days
Untreated soil .....	9.3	36
Toluened soil .....	3.6	73
„ „ + 0.5% untreated soil .....	—	123
„ „ + aqueous extract containing bacteria from untreated soil .....	—	131

The results with the aqueous extract show that no soluble bacterio-toxin occurs in notable quantity in the untreated soil.

Attempts to find a soluble plant toxin were also fruitless. The extracts were made as before from the sick soils and also from partially sterilised soils. Tomato seedlings were used in the earlier experiments, but they were given up because they proved unsuitable for water cultures; while they lived, however, they fared quite as well in the sick soil extracts as in the others. In later experiments barley seedlings

TABLE VI. *Growth of barley in aqueous extracts of untreated and of partially sterilised "tomato sick" soils.*

Soil SB. All extracts were made with ordinary distilled water, and food solution with water prepared in a silver still.

Soil extracts

	Food solution	Sick soil	Partially sterilised soils		
			Treated with toluene	Heated to 55°	Heated to 100°
Weight of produce—Root .....	·0257	·0215	·0205	·027	·0225
Shoot .....	·099	·046	·055	·058	·057
Total, grms. ....	·1247	·0675	·0755	·085	·0795
Length of root, cms. ....	30.2	31.1	31.5	29.0	32.1
Length of shoot, cms. ....	37.2	31.9	35.1	33.3	35.7
Experiment repeated later with the same soil—					
Weight of produce—Root .....	·0345	·030	·024	·030	·023
Shoot .....	·117	·063	·054	·070	·075
Total, grms. ....	·1515	·093	·078	·100	·098
Length of root, cms. ....	40.2	29.1	30.6	32.8	30.6
Length of shoot, cms. ....	37.1	29.2	29.0	33.9	33.8

were used and these made satisfactory growth; again, however, we could find no consistent differences between the plants in the extracts of the untreated, and of the partially sterilised soils. The composition of one of the extracts has been already given: some of the crop results are given in Table VI, while the plants are shown in Pl. V, fig. 4. A large number of other experiments have been made but the results were always negative.

### *Vegetation Experiments.*

(a) *Cucumbers.* Having now shown that "sickness" is to be attributed to a low bacterial efficiency, and that it can be remedied under laboratory conditions by partial sterilisation, it remained to see if similar results could be obtained under the actual conditions of a commercial glasshouse, or whether other factors come into play to obscure these effects. Permission was therefore obtained from a large commercial grower, who has kindly taken very great interest in this work, to conduct an experiment in one of his cucumber houses. One section of the house was made up with old "cucumber sick" soil and another with the same soil heated by steam to 95–98° for four hours. One set of cucumbers was sown in boxes on Feb. 29th, and another set on March 4th, in both cases the plants on the sterilised soil came up later than those on the untreated soil and were at first inferior to them, but afterwards they went ahead and surpassed them. The plants were set out in the borders early in April and soon began to make rapid growth, those in the sterilised soils being much better than those on the sick soil. Yet the latter showed no signs of disease: their leaves were smaller and lighter in colour, but there was no club, wilt or any obvious insect or fungus pest. On May 6th fruit was cut from the plants on the sterilised soil, but none was ready on the plants on the unsterilised soil. The heating had, in fact, cured the "sickness" and rendered the soil commercially profitable once more.

Pl. III, fig. 2 shows a view of the house; typical plants were lifted and found to weigh:

	Green weight, grams	Dry weight, grams
On untreated sick soil ... ..	3025	158·7
On partially sterilised sick soil ...	3731	200·0

Samples of soil were taken from the sterilised and the untreated borders and were subjected to bacteriological and chemical examinations. Owing to the large proportion of dung present it is impossible

to obtain uniform and representative samples, but a general comparison can still be made:—

	Moisture per cent.	Bacterial numbers, millions per gram of dry soil		N per million of dry soil as	
				Nitrate	NH <sub>3</sub>
	May 14	April 17	May 14	May 14	May 14
Untreated sick soil ..	45	174	313	10.4	23.6
Heated sick soil .	46.4	374	735	7.0	15.8

On both occasions the bacterial numbers are much higher in the heated than in the untreated soils. The amounts of nitrates and ammonia in both soils are low compared with the quantities recorded in Table II, an indication that absorption by the plant is well maintained, but it is rather more complete on the heated soil, as might be expected from the remarkable development of fibrous root.

(b) *Tomatoes*. A much more extended series of experiments was made with tomatoes since these plants do not require as high a temperature as cucumbers, and can conveniently be grown in an experimental house. Sick soil was obtained in sufficient quantity to enable us to carry the plants through to the end.

The seeds were sown in small pots (60's), the young plants were thinned out as soon as necessary and transplanted into larger pots (32's), then on to 10 inch pots (16's). During the growth of the plants certain qualitative differences were observed which are dealt with in another paper. The tomatoes were collected and weighed, and, at the end, the plants were lifted and weighed. Determinations were also made of the nitrogen in the plant, and of the nitrates left behind in the soil. It was thus possible to ascertain how far the soils in the greenhouse behaved like those in the laboratory experiments. The results are given in Table VII.

No strict comparison is possible between these values and those recorded in Table V, partly because of the difference in conditions and, partly also because a certain amount of water always runs through the pot during the watering of the plants, and carries with it soluble nitrates. Moreover, in 1911 the plants were not re-potted sufficiently quickly and thus they received rather serious checks. In 1912 we had gained more experience of the crop and were able to remedy this defect, so that we consider these results altogether more reliable. In these



TABLE VII. *Dry matter and amounts of nitrogen in tomato plants grown in untreated and partially sterilised "tomato sick" soils.*

1911 results. Dry matter obtained in root, shoot and fruit, grams.

	Soil MT	Soil MC	Soil SC	Cucumber sick soil B
Untreated soil .....	66.88	30.50	58.31	40.04
Soil heated to 98° .....	103.50	62.49	80.00	75.27
" " + basic slag .....	—	67.89	—	78.76
" " 55° .....	64.77	37.24*	57.40	45.00
Soil treated with 0.5% toluene ..	77.26	44.55	—	51.22
" " CS <sub>2</sub> ..	76.65	44.29	—	46.80

Nitrogen in root, shoot and fruit, grams.

Untreated soil ..	0.947	0.437	0.835	0.645
Soil heated to 98° ...	1.484	0.879	1.313	1.168
" " + basic slag ..	—	0.943	—	1.328
" " 55° ..	0.866	0.594	0.8.1	0.606
Soil treated with toluene . .	1.186	0.678	—	0.703
" " CS <sub>2</sub> .	1.251	0.628	—	0.758

In this case the temperature of heating was 15° only.

1912 results. (Plants cut early.)

	Dry matter in shoot and root, grams	N in root and shoot, grams	N left as nitrate in soil, grams	Total N converted into nitrate and NH <sub>3</sub>	
				grams	parts per million of dry soil
Untreated soil .....	7.1	0.207	0.098	0.340	58
Soil heated to 98° ..	26.8	0.674	0.085	0.789	131
Soil treated with formaldehyde ..	16.6	0.369	0.068	0.465	82
" " pyridene..	13.2	0.419	0.301	0.756	126
" " CaS.....	13.2	0.352	0.053	0.435	72
" " petrol .....	12.1	0.298	0.057	0.402	68
" " toluene .....	11.8	0.295	0.101	0.431	74
" " phenol .....	9.0	0.218	0.091	0.339	58

experiments the amounts of nitrogen obtained by the plant from the partially sterilised soils correspond fairly closely with the laboratory results, an indication that no notable disturbing factor comes into play in the pots.

In at least two cases, however, a serious discrepancy was observed between the action of toluene under laboratory conditions and its behaviour in the pot experiments; the increased rate of decomposition

was induced in the laboratory but not in the pots. The figures for these two soils are:

	Soil <i>MT</i>		Soil <i>A</i>	
	Ammonia and nitrate produced		Ammonia and nitrate produced	
	In Laboratory expts. (Table V)	In pots	In Laboratory expts.	In pots
Untreated soil ...	100	100	100	100
Toluened soil ...	143	104	125	99

The difference was traced to the low solubility of the toluene vapour. In the laboratory experiments the soil is sifted very finely and is confined in small quantities (800 grams) in closed bottles for 36–48 hours while the toluene acts. In the pot experiments this fine sifting is impracticable because it would lead to serious panning; coarse lumps are always present, and the treatment with toluene is carried out in large pots so that the extent of penetration is much reduced. Chemical analyses of soils treated in this way showed that nitrification was checked and ammonia accumulated, but there was no increase in the rate of decomposition. Two soils were found to contain in parts per million:

	Soil <i>RC</i> , 30% moisture			Soil <i>A</i> , 32% moisture		
	Ammonia, after 62 days	Ammonia and nitrate, after 62 days	Relative quantities	Ammonia, after 84 days	Ammonia and nitrate, after 84 days	Relative quantities
Untreated...	7	156	100	9	312	100
Imperfectly toluened )	71	165	106	74	306	98

Our experiments show that toluene acts best on finely sifted fairly dry soils, and loses much of its effectiveness in rich soils when too much moisture is present, or the particles are too coarse.

*The population of the untreated and partially sterilised "sick soils."*

Examination of the sick soils at various times has shown the presence of the following groups of organisms (p. 108):

## Investigations on "Sickness" in Soil

	Untreated soil	Soil heated to 98° C.	Soil heated to 55° C.	Treated with toluene	Treated with carbon disulphide
Fauna	Protozoa (various)*	None	—	Certain flagellates present, others killed	Certain flagellates present, others killed
	Rotifers	"	None	—	—
	Eelworms—Heterodera	"	"	Considerably reduced	Considerably reduced
	Free living forms	Killed but were reintroduced later†	Killed but were reintroduced later	Rarely present but reintroduced later	Killed but were reintroduced later
	Enchytraeid worms	None	None	Present	Present
	Earthworms	"	"	—	—
	Woodlice	"	"	Some killed, others driven away	None
	Millipedes— <i>Julius terrestris</i>	"	"	Present	—
	" <i>pulchrellus</i>	"	"	—	—
	<i>Polydesmus complanatus</i>	"	"	—	—
	Centipedes	Introduced later	Introduced later	Introduced later	Introduced later
	Springtails	None	None	—	—
	Mycetophylid larvae	"	"	Present	None
Flora	Wireworms	"	"	As bad as in untreated	As bad as in untreated
	<i>Pythium de Baryanum</i>	No damping off. Much <i>Pyromonema glaucum</i> (Bondari)†	Rare	—	—
	Plants readily damped off	—	Occasional damping off	—	—
	Fusarium sp.	—	—	Fusarium sp.	Fusarium sp.

\* Some of these have been described by Mr T. Goodey in *Proc. Roy. Soc.* 1911, 84 B, 165—180 and by Mr C. H. Martin in *Proc. Roy. Soc.* 1912, 85 B, 393—400.

† Kindly identified for us by Mr Carleton Rea.

The plants on the "sick" soil are liable to damp off in early life and to be attacked by *Heterodera radicicola*, but "sickness" is not conditioned by these circumstances. Cases have come within our knowledge where plants on sick soil escaped these pests and yet showed the characteristic debilitated state. Our experimental tomatoes suffered from no obvious fungoid disease, probably through the mere accident that the house is well isolated, for the plants on the sick soil looked as if they would take any disease that happened to be about. This weakened physiological condition of the plants on the sick soil no doubt accounts for the close connection between soil sickness and incidence of disease.

*The commercial treatment of sick soils.*

The present method of dealing with cucumber sick soil is to throw it away at the end of the season, or else to carry it back to the field, sow it down with grass, and leave it alone for a few years, then once more bring it back to the cucumber house. This method is so wasteful that any alternative deserves consideration. Our experiments have shown that partial sterilisation affords a satisfactory method of treatment, and trials made under our observation in commercial glasshouses show that it is also practicable on the large scale.

Two general systems may be adopted: the soil may be heated to 90–100° C. or it may be treated with some antiseptic. These systems are fundamentally different. At 90–100° a certain amount of decomposition takes place with formation of products having important secondary effects on the plants which will be discussed in a later paper. Treatment of the soil with antiseptics causes much less decomposition, but certain secondary effects are seen in this case also. Thus in discussing methods of treatment capable of application on the large scale regard must be had not only to the cost and practicability, but also to

- (1) the effect on the bacterial activity in the soil,
- (2) the effect on disease and parasitic organisms,
- (3) the secondary effect on the plant.

Of all methods we have tested so far heat is much the best, but the present cost of heating one ton of soil is about 1s. to 1s. 6d. At this price the process is applicable for growing cucumbers, tomatoes in pots, and certain other glasshouse crops. Chemical treatment is much cheaper and promises greater possibilities of development. Any antiseptic may be expected to serve, but there are two conditions that

must be fulfilled in practice: (1) the antiseptic must be sufficiently soluble to get about in the soil and penetrate the particles, (2) either it must be volatile or it must decompose with formation of innocuous compounds after its work is done, so that it shall not exert any permanent harmful effect on the plant or the food-making bacteria. A large number of substances fulfil these conditions, and the question of selection resolves itself into a comparison of the cost and the effectiveness in the three directions above mentioned. Toluene, which we have used in our laboratory experiments, is unsuitable for commercial work. Apart from the difficulty of transport it is not sufficiently soluble to penetrate the soil particles if much moisture or organic matter is present. Formaldehyde gives very good results and has the great advantage of solubility. Certain tar oils are also very promising, and these when insoluble can be made miscible with water by bringing the specific gravity to 1. In dealing with any commercial product, however, it is always necessary to know the behaviour of the separate constituents in the pure state. An illustration was afforded by commercial toluene. Our most recent pot experiments with pure toluene have given us less satisfactory results than the earlier experiments; other cases have also come to our notice where toluene has behaved in rather a varying manner, sometimes giving fairly large increases and sometimes not. Dr Hodgkinson suggested to us that the sulphur compounds commonly present in toluene (thiophen and thiotolen) were probably the cause of the irregularities; until recently even the purest toluene sold by the dealers contained these sulphur compounds, but lately the specifications of the chief purchasers have been altered so as to require their removal. Thiophen is unfortunately very costly, but Dr Hodgkinson kindly gave us a large supply that he had prepared himself, and we tried its effect on the soil. It proved to be much more active than pure toluene, as shown in Pl. V, fig. 5; indeed pure toluene did not produce any marked effect in this experiment, probably because of its low solubility.

We have had a similar experience with one of the bye-products obtained during the isolation of naphthalene from tar. This particular oil proved to be distinctly useful, and we accordingly fractionated it to get out its chief constituent in a tolerably pure state. But this purer substance was much less useful than the crude oil, and it was clear that the most potent constituent was present in relatively small amounts.

Examination of various waste products and their pure constituents

is in hand. In the meantime we cannot too strongly insist that no waste product should be recommended as a means of treating soil sickness until it can be obtained to a definite specification, and the behaviour of its separate constituents is known.

### *Conclusions.*

1. Sickness in glasshouse soils is conditioned by at least two factors:

- (a) an accumulation of insect and fungoid pests,
- (b) a lowered bacterial efficiency.

2. The lowering of the bacterial efficiency is due to the accumulation of a factor detrimental to bacteria.

3. The sick soils examined did not appear to contain any substance toxic to plants or bacteria. The soils were well supplied with plant food and with calcium carbonate.

4. The factor detrimental to bacteria resembles in every way that present in ordinary arable soil. It is put out of action by heat or by antiseptics. It is not associated with the bacteria but with the soil, and is capable of growth when introduced into partially sterilised soil. In all respects its properties agree with those of protozoa.

5. There is no evidence that sickness is due to an accumulation of bacteria acting unfavourably on the production of plant food (*e.g.* denitrifying bacteria) or that the beneficial effect of partial sterilisation is due to the destruction of such bacteria. So far as we can find all bacterial actions are accelerated in partially sterilised soils; there is, for example, a marked increase in the rate of loss of nitrogen.

6. Soil sickness in tomato and cucumber houses can be effectually treated by partial sterilisation.





Fig. 1. Tomato plants grown in untreated and in partially sterilised tomato sick soils.

The treatment was effected by adding antiseptic equal to 0.25 % of the weight of the soil (except in the case of formaldehyde when 0.1 % was used), leaving it in the soil for 2 days and then allowing it to evaporate before the soil was used. The heating was effected by steam and was continued for 3 hours.







Heated Untreated

Fig. 2. Cucumbers grown in partially sterilised and in untreated cucumber sick soil.  
The partial sterilisation was effected by steam heat continued for 3 hours.

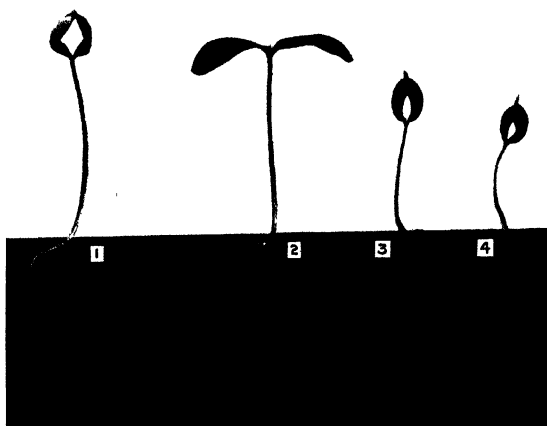


Fig. 3 a. Cucumbers grown in water cultures. 5 days' growth.

- (1) Normal food solution.
- (2) Water extract of cucumber sick soil.
- (3) Water extract of cucumber sick soil made after the soil had been heated for 3 hours to 98° C. by means of steam.
- (4) Extract made after the sick soil had been treated with 0.5% of toluene.



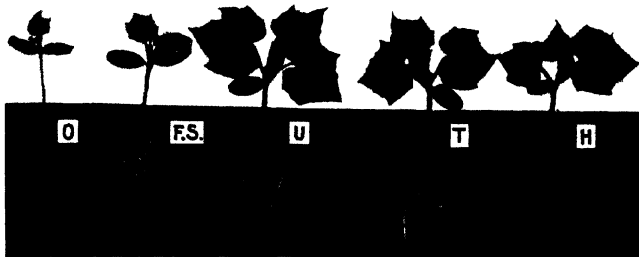


Fig. 3 b. Cucumbers grown in water cultures. 5 weeks' growth.

- O. Tap water alone.      F.S. Normal food solution in tap water.
- U. Extract of cucumber sick soil in tap water
- T. Extract made with tap water after the soil had been treated with 0.5% of toluene.
- H. Extract made with tap water after the soil had been heated for 3 hours to 98° C. by means of steam.

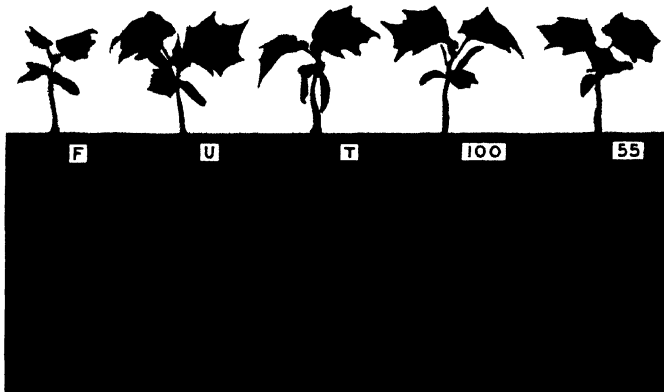


Fig. 3 c. Cucumbers grown in water cultures. 6 weeks' growth.

- F. Normal food solution in ordinary distilled water.
- U. Extract of cucumber sick soil in ordinary distilled water.
- T. Extract made after the soil had been treated with 0.5% of toluene.
- 100. Extract made after the soil had been heated for 3 hours to 98° C. by means of steam.
- 55. Extract made after the soil had been heated for 3 hours to 55° C.



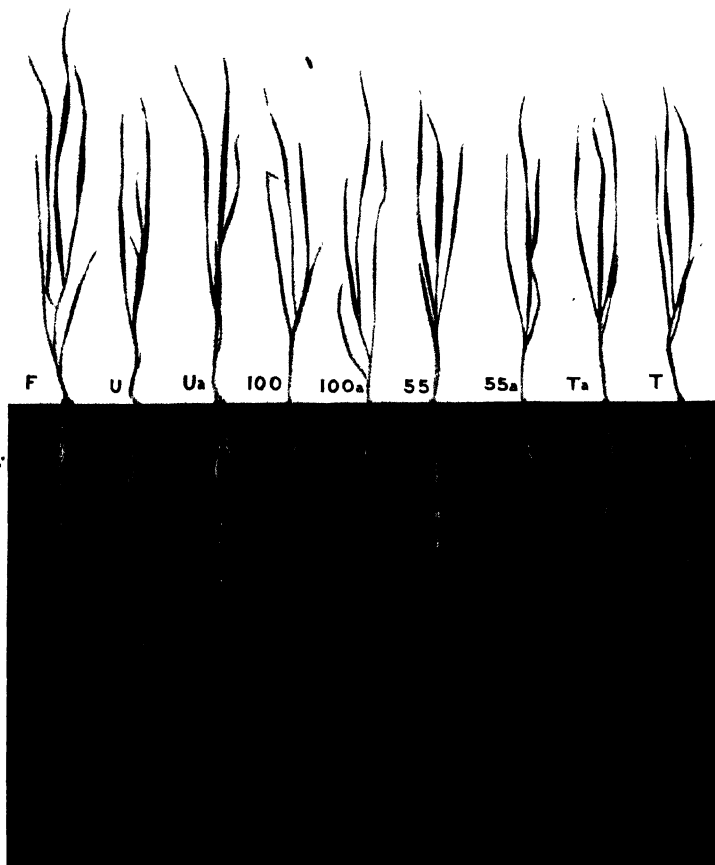


Fig. 4. Barley grown in water cultures.

F. Normal food solution. The others are extracts of tomato sick soil, untreated (U) or partially sterilised (100, 55, T—these having the same meaning as in Fig. 3c). The letter *a* indicates that the extract has been boiled for  $\frac{1}{2}$  hour, a treatment which is seen to have little or no effect in this experiment.



Fig. 5. Young tomatoes growing in tomato sick soil.

Pure toluene has had but little effect in this experiment but thiophen is much more potent.



## LINSEED MUCILAGE.

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THE vegetable mucilages have been, as a whole, but little investigated, probably owing to the difficulties attending their preparation in anything like a state of chemical purity. Workers who have turned their attention to these substances have generally contented themselves with a simple chemical investigation and have left practically untouched the question of the fate of these substances in the animal organism. Thus, amongst other workers, Harlay<sup>1</sup> investigated the mucilage of prickly pear, v. Bittó<sup>2</sup>, the mucilage of capsicum seed, Gaus and Tollens<sup>3</sup> quince mucilage, Yoshimura<sup>4</sup>, the mucilages from several plants, while Hilger<sup>5</sup> examined the mucilage of linseed. With the exception of the latter the work is frequently merely the identification of one or more sugars produced on hydrolysis, with, perhaps, a determination of pentose and hexose sugar-producing complexes. One is struck by the great variation reported between different mucilages. Not only do the sugars produced vary both in nature and quantity, but both the presence and absence of products of hydrolysis other than sugars are reported, while in some cases the original compound is stated to be capable of combining with bases and in other cases to be neutral in character. Probably the compounds, which have been designated mucilages, should not be included in one chemical class, for they have been more frequently characterised by the physical properties of their solutions than by their chemical constitution. Cross and Bevan, however, in their work on "Cellulose," make two definite classes, one, the true mucilages, or muco-celluloses, which yield nothing but sugars on hydrolysis, the other, the pecto-celluloses, which give acid hydrolysis products as well

<sup>1</sup> *J. Pharm. Chim.*, 1902 [vi], 16, 193—198.<sup>2</sup> *Landw. Versuchs-Stat.*, 1895, 46, 1309.<sup>4</sup> *Bull. Coll. Agric.*, Tokyo, 1895, 2.<sup>3</sup> *Ann.*, 249, 245.<sup>5</sup> *Ber.*, 1903, 36, 3197—3203.



as sugars. In the present state of knowledge of the mucilages it is difficult to say whether such a classification will finally hold or not, for, owing to the difficulties in purifying mucilaginous substances, it is almost impossible to say whether the substance dealt with is a single chemical individual or a mixture. But in a case like linseed mucilage, where exceedingly large quantities are consumed every year by cattle and sheep, whether the substance, which, when purified certainly behaves as though it contains a very greatly preponderating quantity of one chemical individual, be a single substance or not, a knowledge of its chemical composition and fate in the animal organism will certainly be valuable, and the present work was undertaken from that point of view.

Bauer<sup>1</sup> had already identified dextrose in the products of hydrolysis. Kirchner and Tollens<sup>2</sup> state that they obtained arabic acid and cellulose on hydrolysis, while Hilger<sup>3</sup> investigated the substance much more fully and the main facts worked out by him were confirmed and are noted in the text.

The work naturally divided itself into the purely chemical investigation, and the examination of the substance as a feeding stuff; and in that order the experimental work is given below.

#### PREPARATION OF THE MUCILAGE.

Two methods for the extraction of mucilage from the seed were tried; extraction by 1 per cent. sulphuric acid in the cold and extraction with cold water only. The solvent was, in each case, allowed to act on the linseed for 24 hours, when the mucilaginous liquid was squeezed away from the seed through fine linen, and the process then repeated until very little mucilage could be obtained from the extract. The extracts so obtained contained a small amount of flocculent matter which was allowed to settle and the mucilage solution decanted therefrom, filtration being so slow as to be impracticable. Both extracts were then treated with phosphotungstic acid solution (2.5 grs. phosphotungstic acid and 5 grs. sulphuric acid in 100 c.c. water) until no further precipitate was obtained. The water extract gave only a slight precipitate with the reagent, while the acid extract gave a voluminous precipitate. As this indicated that the extraction of proteid matter was greater in the case of the acid solution, this method was discontinued and the water extract alone used.

<sup>1</sup> *Landw. Versuchs-Stat.*, 1892, 40, 480.

<sup>2</sup> *Ann.* 175, 205.

<sup>3</sup> *Ber. loc. cit.*

The mucilage solution was now treated with twice its bulk of 90 per cent. alcohol, when the mucilage was obtained as a white fibrous mass, which was dehydrated with absolute alcohol, washed with ether and finally dried in vacuo. The substance so prepared contained 1.5 per cent. ash and 1.8 per cent. proteid matter, the latter being calculated from a Kjeldahl nitrogen determination. The ash content was further decreased by treating the substance with very dilute hydrochloric acid and again precipitating and drying, when the ash content was reduced to 0.6—0.7 per cent. Attempts at further purification by again acidifying with hydrochloric acid and dialysing did not give results which justified the use of the process on a larger scale. The quantity of mucilage obtained by this method was much smaller than anticipated. In one case 700 grs. of linseed when exhaustively extracted gave 44 grs. of mucilage or 6.28 per cent. Other extractions gave figures agreeing closely with this figure.

#### PROPERTIES OF THE MUCILAGE.

Prepared in the manner described above the mucilage is obtained as a friable white powder which "dissolves" in water to an opalescent "solution" which while neutral to litmus is capable of neutralising caustic alkalis. In an actual experiment 0.334 gm. mucilage required 0.0188 gm. sodium hydroxide for neutralisation to phenol-phthalein, the estimation being made by adding excess of the alkali and titrating back with acid. If the substance is assumed to behave as a monobasic acid this result would give a molecular weight of 710. From the general properties of the substance however this is, without doubt, only a sub-multiple of the true molecular weight. It may be noted that it agrees closely with a molecule four times the size of  $C_8H_{12}O_4$ , which is the empirical formula for the substance given by the combustion results described below. The solutions in caustic alkalis are quite clear and the substance appears to be in true solution. These solutions are dextrorotatory and a determination of rotatory power shewed that 0.2085 gm. mucilage, exactly neutralised with caustic soda, and made up to 25 c.c. with water, gave  $\alpha = +0.09$  in a 1 dm. tube,

whence  $[\alpha]_D = +10.79$ .

Clear solutions may also be obtained in ammonia but in this case considerable excess of the reagent is necessary. With alkali carbonates the mucilage solutions give no reaction.

The substance is slowly hydrolysed by boiling with dilute mineral acids. It is very slowly, if at all, attacked by hot alkali. It gives no coloration with iodine, does not reduce Fehling's solution, and gives no reaction with phenylhydrazine.

Solutions of the salts of the heavy metals give gelatinous precipitates, and the substance itself is insoluble in all the organic solvents.

As extracted from the seed, the mucilage contains a considerable quantity, about 2 per cent., of ash and this contains calcium, potassium, magnesium, iron and phosphoric acid.

The mucilage does not melt below 250° C. and is apparently unchanged by heating for some time at 150° C. though when heating above 200° C. is prolonged for some time it gradually becomes brown in colour and some decomposition takes place.

On combustion it gave the following result :

0.1789 gm. substance gave 0.2753 gm. CO<sub>2</sub> and 0.1030 gm. H<sub>2</sub>O,  
whence C = 41.96 per cent.; H = 6.37 per cent.

(C<sub>6</sub>H<sub>12</sub>O<sub>6</sub>)<sub>n</sub> requires C = 40 per cent., H = 6.66 per cent.,

Although in the carbon estimation there is nearly 2 per cent. difference between the estimation and the empirical formula suggested, it must be remembered that it is impossible, owing to the nature of the substance, to obtain it chemically pure for analysis: the figures given however agree more closely with the suggested formula than with any other carbohydrate formula.

In a Berthelot bomb calorimeter (of water equivalent 333) 0.7642 gm. mucilage, with 0.0145 gm. iron firing wire, gave a rise of 1.298° C. to 2000 c.c. water. The radiation correction was - 0.0024° C.

From the above, the heat of combustion for 1 gm. of the substance is shown to be 3925 calories. This is of the order to be expected from a body of carbohydrate nature and compares with 3866 for cane sugar, 4123 for starch and 4146 for cellulose.

Nitration with concentrated nitric acid was unsuccessful, but the hydrated cellulose nature of the mucilage was very definitely shown by the action of acetyl chloride and benzoyl chloride.

*Acetyl Derivative.* The substance was boiled with excess of glacial acetic anhydride for several hours. The action was very slow but after some time the substance dissolved completely, when the solution was filtered, poured into cold water and well shaken. A white flocculent precipitate was obtained, and this was purified by redissolving in glacial

acetic acid and precipitating by water or ether. The substance so obtained was a white amorphous body, soluble in glacial acetic acid, ethyl acetate and acetone, moderately soluble in a mixture of alcohol and ether, and insoluble in other solvents. Films of the solutions dry down to transparent glassy masses.

The substance melted at  $240^{\circ}$ — $245^{\circ}$  C., with decomposition, and on combustion gave the following figures :

0.1639 gm. gave 0.2837 gm.  $\text{CO}_2$  and 0.0834 gm.  $\text{H}_2\text{O}$ ,  
whence  $\text{C} = 47.20$  per cent.,  $\text{H} = 5.65$  per cent.,

$\text{C}_6\text{H}_9\text{O}_6(\text{CO}\cdot\text{CH}_3)_3$  requires  $\text{C} = 47.06$  per cent.,  $\text{H} = 5.88$  per cent.

*Benzoyl Derivative.* The substance was dissolved in caustic soda solution (actual  $\text{NaOH} = 20$  per cent. of weight of mucilage) and benzoyl chloride added gradually, with constant shaking, until a large excess of the reagent had been used. A flocculent white precipitate was formed, which was filtered off, washed and dried. The substance was thus obtained as a friable white powder melting with decomposition at about  $270^{\circ}$  C. It was insoluble in water and ether, slightly soluble in acetic acid, and to a still slighter degree in alcohol.

0.1327 gm. gave 0.2992 gm.  $\text{CO}_2$  and 0.0620 gm.  $\text{H}_2\text{O}$ ,  
 $\text{C} = 61.49$  per cent.;  $\text{H} = 5.18$  per cent.,

$\text{C}_6\text{H}_9\text{O}_6(\text{CO}\cdot\text{C}_6\text{H}_5)_2$  requires  $\text{C} = 61.85$  per cent.,  $\text{H} = 5.16$  per cent.

The formation of these two derivatives shows that the mucilage is very probably a hydrated cellulose, since the typical cellulose itself behaves in a similar manner with acetyl and benzoyl chlorides, and shows thereby the presence of hydroxyl groups, two of which are situated differently from the third, in the simple molecular unit.

Although from its general behaviour as a colloid it was anticipated that no molecular weight determinations on the mucilage would be possible, the attempt was made with Walker and Lumsden's modification of Landberger's boiling point apparatus. The results were only negative, the minute changes of boiling point noticed being easily accounted for by the small ash content of the substance.

#### HYDROLYSIS OF THE MUCILAGE.

When boiled for some hours with dilute mineral acids the mucilage is completely hydrolysed, though the process is slower than was anticipated. Dilute sulphuric acid (4 per cent.) was used, and the dark brown

solution obtained after six hours boiling was filtered free from a small quantity of flocculent matter, treated with animal charcoal, filtered and baryta added until just alkaline. The barium sulphate was filtered off and to the clear solution alcohol added in considerable quantity. There was thus precipitated a small quantity of amorphous white substance containing a considerable quantity of barium which was filtered off from a clear yellowish solution. The alcohol was distilled off from the solution and the three products (*a*) the flocculent matter obtained on first hydrolysing, (*b*) the solid precipitated by alcohol, and (*c*) the clear aqueous solution, examined separately.

(*a*) This product is small in quantity, is of a humic nature and very probably not a hydrolysis product of the mucilage itself since, with the purest samples of mucilage used, the quantity formed was practically negligible, while with cruder samples much larger quantities were obtained. The author has little doubt that this product would be entirely absent if it were possible to work with chemically pure mucilage.

(*b*) This substance appeared at first to be the barium salt of an organic acid. It was dissolved in water, sulphuric acid added till all the barium was precipitated and the barium sulphate filtered off. The solution remaining was evaporated to small bulk and the last traces of water taken off in a vacuum desiccator. The material so obtained was found to contain phosphoric acid. This was removed as calcium phosphate and the other acid obtained in the form of its calcium salt. This substance on ignition gave in two cases 16.62 per cent. and 16.93 per cent. CaO.

The calcium salt is amorphous, easily soluble in water and strongly dextrorotatory.

0.249 made up to 25 c.c. with water gave  $\alpha = +1.3$  in a 2 dm. tube, whence  $[\alpha]_D = +65.26$ .

On combustion of a specimen of this calcium salt the following results were obtained:

0.1704 gm. gave 0.2032 gm. CO<sub>2</sub> and 0.0986 gm. H<sub>2</sub>O,

C = 32.51 per cent., H = 6.43 per cent.

The substance contains no nitrogen, does not reduce Fehling's solution, and the free acid dissolved in sodium carbonate solution does not decolorise permanganate solution. Also it does not give, so far as could be detected by the ordinary colour reaction, any furfural on treatment with hydrochloric acid.

The barium and cadmium salts which were also prepared are amorphous and present no advantage for manipulation over the calcium salt. The quantity of this acid product of hydrolysis varies slightly with different samples of mucilage, but more than 2.5 per cent. was never obtained, and this small yield renders the investigation of the substance somewhat difficult.

It may be noted here that, whatever be the constitution of this acid body, it can hardly be, as stated by Kirchner and Tollens<sup>1</sup>, the ordinary form of arabic acid, since this acid is laevorotatory, while the mucilage acid is strongly dextrorotatory. In a general way, the mucilage acid appears to be similar to the geddic acid described by O'Sullivan<sup>2</sup>, and may be produced from a complex carbohydrate in a similar manner to geddic acid from gedda gum.

The author is not convinced that the acid is necessarily a hydrolysis product of the mucilage since the method of extraction of the mucilage does not preclude the presence of other non-nitrogenous bodies in the aqueous extract, and the small and varying quantity which is obtained of this product lends some support to this view.

(c) The aqueous solution has a strong reducing action on Fehling's solution and in a preliminary experiment it was shown that this aqueous solution from the hydrolysis of a known weight of mucilage had practically the same cupric reducing power as an equal weight of hydrolysed starch. The solution was investigated as follows :

(1) To a portion of the solution strong nitric acid was added until the specific gravity reached 1.3, and the mixture was then heated for some time at 60° C. The liquid was then diluted with water and allowed to stand, when a white crystalline precipitate separated out. This was extracted with hot alcohol to remove any oxalic acid present and the residue was recrystallised from hot water. It was thus obtained as a white crystalline powder melting at 225° C. with decomposition, and giving results on analysis corresponding with mucic acid. The substance was therefore mucic acid and the original solution contained galactose.

(2) A portion was oxidised with bromine water at the ordinary temperature, the excess of bromine removed by heating, the solution neutralised by cadmium carbonate, evaporated down, and alcohol added. A white solid crystallising in prismatic needles was obtained. The substance had no definite melting point but an estimation of bromine corresponded with the composition  $C_6H_5O_6.CdBr.H_2O$ , the double

<sup>1</sup> *Ann. loc. cit.*

<sup>2</sup> *J. C. S.*, 1891, T. 1029.

cadmium salt of xylonic and hydrobromic acids, and the formation of this was shown by Bertrand<sup>1</sup> to be characteristic of xylose.

(3) A large portion of solution was treated with phenylhydrazine, and the mixed osazones which separated were collected in two portions, the first being a yellow solid substance and the second a dark brown oil which afterwards solidified. By fractional crystallisation of these from dilute alcohol there were separated the osazones of glucose (M.P. 204° C.), arabinose (M.P. 160° C.) and xylose (M.P. 150—151° C.).

(4) A portion of the solution was treated with diphenylhydrazine and the precipitate produced was purified several times by crystallisation. It showed the characteristics of xylose diphenylhydrazone and melted at 204—205° C.

There were thus identified in the solution the four sugars glucose, galactose, arabinose and xylose which Hilger<sup>2</sup> had noticed.

Experiments were now carried out with a view to obtaining some quantitative determinations of the sugars obtained by hydrolysis. Ten grams of mucilage, containing, when allowance had been made for the water, ash and protein content, 8.2 gm. pure mucilage were hydrolysed with 4 per cent. sulphuric acid. The proteid matter was removed by phosphotungstic acid, the mineral acid precipitated by baryta, and the barium salt of the acid decomposition product precipitated by alcohol. The solution which should now contain sugars only was evaporated to small bulk and the remaining water removed *in vacuo*. There were thus obtained 7.31 gm. of a yellowish solid mass, which was practically ash free, quite nitrogen free, and which, as far as could be shown by qualitative tests, contained sugars only. That is, 89.1 per cent. of the weight of the mucilage taken can be recovered as sugars.

The sugars were then distilled with hydrochloric acid, as for the usual estimation of pentosans, and the furfural distilling over was precipitated as the phenylhydrazone. There was thus obtained hydrazone corresponding to 1.1463 gm. pentose sugar,  $C_5H_8O_4$ . This corresponds to 16.88 per cent. of the total sugars existing as pentoses.

Another furfural determination done directly on 8.204 gm. pure mucilage (when allowance had been made for water, ash and proteid) gave hydrazone corresponding to 1.407 gm. pentose sugar or 17.15 per cent. of the pure mucilage, and this figure confirms the estimation above on the previously hydrolysed material.

<sup>1</sup> *Bull. Soc. Chim.* [3], 7, 499—502.

<sup>2</sup> *Ber. loc. cit.*

The figures above would seem to indicate that practically the whole of the mucilage is hydrolysed to sugars, and that of the sugars about 17 per cent. are pentoses. Since two pentose sugars were identified, an expression such as  $2 \text{C}_6\text{H}_8\text{O}_4 \cdot 6 \text{C}_6\text{H}_{12}\text{O}_6$  would seem to be the simplest way of representing the original constitution of the hydrolysis mixture, since this expression requires 19.6 per cent. pentose sugars, and two of these sugars are present. This differs from the sample of mucilage investigated by Hilger, who found equal quantities of hexose and pentose sugars.

#### SUMMARY OF CHEMICAL INVESTIGATION.

The preceding experiments show that linseed mucilage is a carbohydrate body showing all the characteristics of the hydrated cellulose, and that it is well described by the term "muco-cellulose" under which such substances are classed by Cross and Bevan. It gives on hydrolysis both hexose and pentose sugars in such quantity that, for practical purposes, at any rate, it can be considered to give nothing else. In fact it is very doubtful whether the other products obtained in hydrolysing an average sample are decomposition products or, at any rate, direct, decomposition products of pure mucilage. The author inclines to the opinion that they are not.

#### ACTION OF ENZYMES ON MUCILAGE.

From a practical point of view the possible fate of the mucilage in the animal organism is of considerable importance, and the action of the different digestive ferments on the material was investigated.

In most cases 5 gm. of mucilage were weighed out, dissolved in a considerable quantity of water, the enzyme preparation added and the whole made up to 250 c.c. with water, or smaller quantities were used at the same concentration. The solution was made faintly acid or alkaline as required by the different ferments, and any sugar formed was estimated indirectly by its cupric reducing power, using ferric alum and standard permanganate solution (1 c.c. of the latter being equivalent to .005 gm. dextrose) to estimate the  $\text{Cu}_2\text{O}$  produced. In the estimations the unchanged mucilage was precipitated by alcohol, filtered off, well washed, dried and weighed. The filtrate, which should contain any sugar formed, was freed from alcohol by heating and the sugar estimated as mentioned above. The reaction mixtures were kept at  $41^\circ \text{C}$ . in a thermostat, and in all cases the activity of the



enzyme preparation was tested by trying it on starch under exactly similar conditions.

The ferments were found to be without action on the mucilage solutions, a small initial reducing power of the solution remaining unchanged after many hours. Particulars are given below of some of the experiments, showing the general character of the results obtained.

#### TAKA DIASTASE.

Two grams mucilage in 100 c.c. water. Ten c.c. taken for each estimation.

Sample	Time in hours	Permanganate required to oxidise $\text{Cu}_2\text{O}$ formed	Grams sugar present calculated as dextrose	Mucilage recovered
1	0.75	2.3 c.c.	0.0115	0.1660 gm.
2	1.25	2.6 "	0.0130	0.1686 "
3	4.0	2.5 "	0.0125	0.1775 "
4	6.86	2.6 "	0.0130	0.1762 "
5	23.25	2.7 "	0.0135	0.1689 "
6	192.0	2.7 "	0.0135	0.1654 "

The weight recovered should be, of course, 0.2 gm., but the error in recovering and drying is considerable, and lack of increase in the reducing power of the solution shows that no reaction was taking place.

#### BARLEY DIASTASE.

Two grams mucilage in 100 c.c. water. Ten c.c. taken at a time for estimation.

Sample	Time in hours	Permanganate required to oxidise $\text{Cu}_2\text{O}$ formed	Grams sugar present calculated as dextrose	Mucilage recovered
1	0.33	3.2 c.c.	0.0160	0.1727 gm.
2	0.66	3.0 "	0.0150	0.1686 "
3	1.9	3.4 "	0.0170	0.1630 "
4	7.0	3.1 "	0.0155	0.1640 "
5	26.0	2.7 "	0.0135	0.1685 "
6	53.2	2.4 "	0.0120	0.1605 "

## "ZYMINE."

A commercial preparation of the "digestive principles of the pancreas." Five grams mucilage in 250 c.c. water. Twenty c.c. taken as test portion.

Sample	Time in hours	Permanganate required to oxidise $\text{Cu}_2\text{O}$ formed	Grams sugar present calculated as dextrose	Mucilage recovered
1	0.33	2.7 c.c.	0.0135	0.3879 gm.
2	1.16	3.0 "	0.0150	0.3500 "
3	1.93	2.9 "	0.0145	0.3600 "
4	2.5	3.4 "	0.0170	0.3821 "
5	26.25	3.3 "	0.0165	0.3450 "

## SALIVA.

Five grams mucilage treated with saliva made up to 250 c.c. with water. Twenty c.c. taken for each test portion.

Sample	Time in hours	Permanganate required to oxidise $\text{Cu}_2\text{O}$ formed	Grams sugar present calculated as dextrose	Mucilage recovered
1	1.0	2.0 c.c.	0.0100	0.3620 gm.
2	3.25	2.3 "	0.0115	0.3657 "
3	5.25	2.2 "	0.0110	0.3593 "
4	25.0	2.2 "	0.0110	0.3421 "

## PEPSIN.

Exactly similar results to the above were obtained with pepsin on the mucilage, although control experiments showed the enzyme preparation to be of normal activity. Even after thirty hours 89—90 per cent. of the mucilage could be recovered unchanged.

## EXTRACT OF OX PANCREAS.

The same negative results were obtained with an extract of ox pancreas prepared in the laboratory.

## FEEDING EXPERIMENTS.

The only conclusion to be drawn from the above results was that the common digestive enzymes were without action on linseed mucilage,

and presumably therefore it would pass through the animal, at any rate through the non-ruminants, unchanged. Feeding experiments were therefore carried out with animals.

The first experiments were attempted with guinea-pigs, the animals being fed for one period on green food and afterwards on the same food mixed with linseed. The animals proved unsuitable for the purpose. They did not readily eat the linseed, and it was difficult to devise any method for showing whether or not the mucilage was passing through the animal.

Rats proved, however, to be much more suitable, and an experiment was arranged as follows. Six rats, equally divided between three cages, were kept for a five or six day period on a prepared diet, and were afterwards fed for a similar period on the same diet, except that a certain amount of starch was replaced by prepared mucilage. The carefully weighed-out food was given in such quantity that each rat received about 9 gm. of dry matter per day, and the faeces were collected, dried and weighed. The loss of food and faeces must have been very small, as the open-work wire bottoms of the cages, which were raised above a sheet of white filter paper resting on sawdust, allowed all uneaten food and faeces to be collected easily and accurately, and, as the animals were only allowed a small quantity of cotton wool as bedding, loss therein was negligible. This arrangement of cages also prevented any appreciable admixture of the urine with the faeces, the former being taken up rapidly by filter paper and sawdust.

The tables below give the figures for the experiment and such particulars as are necessary for the working out of the final result.

Period I. Diet (A)		Period II. Diet (B)	
Starch	162.4 gm.	Mucilage	89.5 gm.
Sugar	81.2 "	Starch	81.2 "
Lard	48.8 "	Sugar	81.2 "
Casein	97.6 "	Lard	48.8 "
Ash	10.8 "	Casein	95.1 "
		Ash	5.0 "

(The "mucilage" used in diet (B) contained 5.8 gm. of ash and 2.5 gm. albuminoid matter, so that the second diet was identical with the first except for the replacement of 81.2 gm. starch by an equal quantity of pure mucilage.)

The ingredients were thoroughly rubbed together, made into small cakes with the help of a little water and then heated for a short time in a water oven. After this preparation, cakes from (A) contained 27·22 per cent. of water, while cakes from (B) contained 31·2 per cent. water.

In Period I, Cage I rats were, owing to an accident, only allowed a five-day period, but in all other cases a six-day period was given. The faeces were first collected 18 hours after the first meal was given and up to the same length of time after the last meal. An interval of a week on ordinary diet was given between the two periods.

Cage	Period I.		Period II.	
	Dry matter eaten	Faeces	Dry matter eaten	Faeces
	gm.	gm.	gm.	gm.
I	84·98	4·745	81·02	13·5
II	100·99	5·071	74·64	12·91
III	104·99	5·055	73·45	10·73

#### HEAT OF COMBUSTION OF FOOD AND FAECES.

*Small calories per gram of dry matter.*

Period I.		Period II.	
Food	Faeces	Food	Faeces
4960	4494	4785	4846

The heat of combustion of the sample of "mucilage" used was 3725 calories per gram.

From the above tables the following statement can be made out, taking the average value per rat per day.

*Values per rat per day.*

		Period I.	Period II.
Calories in food eaten	...	42408	30452
" faeces	...	1966	5001
Per cent. of energy in faeces		4·64	16·42

The difference in the energy lost in the faeces in the two periods is 11·78 per cent. From the figures given it can be calculated that the mucilage provided 15·6 per cent. of the energy of the food.

It is difficult to suggest the probable experimental error in an experiment such as this, but the figures point to very slight utilisation of the mucilage by the animal. It can be stated with certainty that 75 per cent. of the mucilage passes through the animal unchanged, and

even the appearance of the faeces showed the presence of a considerable quantity of unchanged mucilage. It was still, however, possible that the linseed itself carried an enzyme which would cause the break-up of the mucilage when the latter was eaten in the ordinary way as part of the seed, and that the enzyme was absent in the diet given in the experiment above. A preliminary experiment on this point had already been done when carrying out the thermostat experiments with the digestive enzymes, a solution of the mucilage being treated with cold water extracts of both germinating and resting seeds. No action could be detected, but it was just possible that the seed contained an enzyme which only became active in the alimentary canal of the animal, as in the case recorded by H. Brown<sup>1</sup>.

To test this point, a sample of linseed, containing 5.4 per cent. water, 24.25 per cent. proteid and 37.50 per cent. fat, was finely ground and made into small cakes with starch, sugar and casein in the following proportions :—

Linseed	220	gms.
Starch	200	„
Sugar	200	„
Casein	120	„

The food thus prepared was divided into two portions, one dried at the ordinary temperature and one heated to 110° C. for 15 hours in order to destroy any enzyme present. The quantity of food given and the faeces collected are given in the following table, the weights being the average ones per rat per day.

	Dry matter eaten	Dry matter in faeces	Calorific value of food	Calorific value of faeces
Heated food	7.886	0.828	4817 cal. per gm.	4909 cal. per gm.
Unheated food	8.312	0.911	4694 „ „	4666 „ „

From the figures given above the following comparison of the two periods is obtained.

*Calories per rat per day.*

	In food	In faeces	Per cent. calories in faeces
Heated food	37986	4064	10.7
Unheated food	39016	4250	10.9

There is thus shown to be practically no difference in the two cases, and it can be assumed that the seed does not carry any enzyme capable of breaking down the mucilage in the animal organism.

<sup>1</sup> J. C. S., T. 1892, 527.

In order to confirm the above experiment, it was repeated with prepared mucilage added to the diet in addition to the linseed itself. In this way the percentage of mucilage in the diet was raised to 8 per cent., and any difference in utilisation in the two periods would probably be more marked than in the previous experiment, but no difference could be detected.

#### BACTERIAL ACTION.

There still remained the possibility that in the ruminants, where bacterial digestive action is great, the mucilage might be more completely broken down than in other animals. Unfortunately the preparation of mucilage on a sufficiently large scale to form any appreciable portion of a ration for a sheep or cow is impossible with only laboratory apparatus, but an attempt was made to obtain some information by laboratory experiments.

To a dilute solution of the mucilage, kept in a thermostat at 38° C., was added a small quantity of fresh caecum contents from a cow. The flask containing the mixture was arranged so that any gas evolved could be collected. In the first experiment from 2 gm. of mucilage in 250 c.c. water containing nutrient salts there were obtained about 70 c.c. of gas, of which 50 c.c. were absorbed by potash and the remainder was inflammable. The evolution of gas, however, quickly stopped, and when the contents of the flask were tested they were found to be strongly acid. The experiment was therefore repeated with the addition of a quantity of calcium carbonate to the solution. In this case the gradual evolution of gas continued for a long period and in the course of a few hours 500 c.c. of gas were easily collected. About 90 per cent. of the gas was carbon dioxide, about 6 per cent. methane and the remainder oxygen and nitrogen. From the contents of the flask about 7 per cent. of the weight of mucilage taken could be distilled over as volatile acids when heated with dilute sulphuric acid, and in this distillate butyric and acetic acids were recognised. In the reaction flask there is also precipitated from the clear mucilage solution a certain amount of flocculent matter, and even after some time small quantities of mucilage could be precipitated by alcohol.

Attempts were made to use the mucilage for bacterial culture, like agar-agar on Petri dishes, to differentiate if possible between those bacteria which attack the mucilage and those which do not. The attempt was not successful, for it was found to be impossible to keep the mucilage at the right stage of hydration on the plate.

## SUMMARY OF ACTION OF ENZYMES, ETC.

Under laboratory conditions the mucilage is unattacked by the digestive enzymes, and even when fed to a non-ruminant animal 75 per cent. can be shown to pass through unchanged. As far as such a point can be demonstrated by laboratory experiments, it is however probable that the mucilage is attacked by intestinal bacteria, and, in ruminants especially, largely broken up in this way, with the evolution of gases and certain volatile acids among other products. This behaviour while unexpected at the beginning of the investigation is not inconsistent with its chemical character as a hydrated cellulose. A similar case is perhaps found in the substance agar-agar, which is readily attacked by bacteria, but is stated by Armstrong in his article on "Carbohydrates" in Allen's *Organic Analysis* to be practically indigestible by the human organism.

The above experimental results draw attention once more to the use of the term "soluble carbohydrates" in connection with feeding stuffs. In the usual routine analysis of foods many different compounds are grouped under this heading and are necessarily assigned one feeding value. Where the sugars and starches form the great bulk of the substances so grouped no great error results; but where, as in the case of linseed, the principal "soluble carbohydrate" is one of very different behaviour in the animal organism, the ordinary analysis may be misleading. Although it was impossible to prepare mucilage in sufficiently large quantities to carry out rigid feeding experiments on large animals, the results which were obtained point to a much lower actual feeding value for linseed mucilage than for starches and sugars, and this result is of considerable importance since popular opinion assigns to linseed such a high value, and even routine analysis necessarily gives it a value equal to the sugars and starches.

The best thanks of the author are due to Professor T. B. Wood for many valuable suggestions and for the interest he has taken in the work throughout.

## PASTURE PROBLEMS: DROUGHT RESISTANCE.

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OWING to the exceptional drought of 1911, an excellent opportunity presented itself for studying the behaviour and power of resistance of the several species which contribute to the herbage of pastures and meadows. The very variable nature of the soils and pastures, which forms a striking and perplexing feature in regard to agricultural problems on the Cotswolds, contributes to make the area eminently suited for an investigation of this sort.

Quantitative analyses were made on a number of typical fields at different dates as the season advanced with a view to tracing the progressive relation between climatic conditions and the personnel of the several pastures. The results obtained from a small series of analyses have been published elsewhere<sup>1</sup>. It is now proposed to treat the question more fully in the light of a larger series of analyses which carries the investigation to the end of April 1912, the condition of the herbage in the spring being found to afford a valuable commentary on the effect of the drought.

### *Area Investigated: Soil.*

The fields investigated were selected so as to exemplify the most frequent types of grass land met with on the Cotswold area. Most are on the farms of the R.A. College, Cirencester, of Mr Bruce Swanwick, Coates, at an elevation of about 430 feet above sea level. The soil in the case of these is derived from the Great Oolite, being a dry calcareous loam (10%—14% calcium carbonate) varying in depth from 6" to 24". The subsoil, except where otherwise stated, is brashy calcareous rock (15%—40% calcium carbonate). The results obtained from a few fields differently situated are given in some instances for the sake of

<sup>1</sup> Stapledon, R. G., "The effect of the Drought of 1911 on Cotswold Grass land." *Roy. Agric. College, Cirencester, Scientific Bulletin*, No. III. for 1911.



comparison. Really first-class grass land does not come under the scope of the present inquiry.

*Type Fields: Particulars*<sup>1</sup>.

(a) FIELDS ON R.A. COLLEGE FARM.

A. No. 15.

Put down to grass about 1894 after sainfoin, with a mixture of the finer grasses and Dutch clover. It was renovated about six years ago with a sowing of perennial rye grass and Dutch clover. It is typical of much of the prepared sheep walks of the district and normally affords abundant keep. It was grazed heavily all through the summer with sheep and cattle.

B. No. 13 (*the Chapel Close*).

Put down to grass over fifty years, it now consists largely of endemic species, and is typical of meadow land on the thin soils. It maintains a high average yield of somewhat coarse hay. It was recently liberally dressed with farmyard manure and artificials.

C. No. 16.

Put down in 1895 with a mixture of meadow foxtail, cocksfoot, tall fescue, meadow fescue, perennial rye grass, rough-stalked meadow grass, alsike, red and white clovers. A high exposed field, periodically cut for hay. The soil, although shallow, contains a considerable amount of clay.

D. No. 12.

Under grass since 1862. It has been liberally dressed and mown constantly since 1888 with occasional two or four years rests. Typical of the prepared meadows on the deeper soils, maintaining an average of about 25 cwt. of hay to the acre.

E. No. 14.

Permanent pasture since 1892, carries sheep and cattle to which extra foodstuffs are fed.

<sup>1</sup> The capital letters serve as references to the Tables. Particulars as to soil-depth and calcium carbonate are shown in the Tables.

*Portion (a).* Owing to its position this part of the field receives considerable supplies of water by percolation from the College drainage system above. The herbage was consequently well maintained all through the summer, but was coarse.

*Portion (b).* Typical of the better classes of grazed pasture on the deeper soils.

(b) FIELDS SITUATE OTHERWHERE THAN ON THE R.A.  
COLLEGE FARM.

F. *Barren Rabbit Warren at Colesborne.*

This field is typical of much waste land in the Cotswolds occurring at elevations of 630 feet and upwards above sea level. It consists of a natural herbage which in the past has carried rabbits only. The soil is derived from the Inferior Oolite and ranges from 3" to 6", seldom attaining to 9" in depth, and contains from 20% to 34% of calcium carbonate and is singularly bare of vegetation.

G. *Old Permanent Pasture at Latton, Cricklade.*

Rough herbage grazed by cattle; soil stiff alluvium resting on calcareous gravel.

H. *Old Permanent Grass at Dry Leaze, Cirencester.*

Gives hay of poor quality. Soil clay (about 1%  $\text{CaCO}_3$ ), due to leaching out of calcium carbonate from Oolite soil cap.

I. *Irrigated Water Meadow, North Cerney, Cirencester.*

Very productive well-managed meadow. Since the conditions are the direct opposite to those obtaining on the thin soils under investigation, the field is peculiarly valuable for the sake of comparison.

K. *Four and Two Year Old Leys, Coates.*

Full particulars of the mixtures used are available and are given hereafter (p. 138).

## BOTANICAL ANALYSES: METHODS.

(a) *Qualitative Analyses.*

A preliminary examination was made on each of the fields selected. The plan usually employed was that previously adopted on arable land<sup>1</sup>.

*Specific Frequency*<sup>2</sup>. A number of readings are taken with a mesh 6" × 6". In the case of each reading the species present are noted without regard being paid to the individual frequency of the plants. The results are conveniently shown by the number of times each species occurs per 100 readings or by merely drawing up a list of the species in order of their relative frequency. This plan gives a good idea of the general character of a field and greatly facilitates the selection of typical plots. Since the analyses now under consideration were made for the special purpose of comparison at relatively frequent intervals typical plots of about 1/10th of an acre were chosen for each field and the samples for all the analyses taken from them.

(b) *Quantitative Analyses.*

Two methods have been employed :

(1) *Number of Plants to the Acre: Percentage Frequency.* These analyses were made by counting the individual plants in the manner prescribed by Armstrong<sup>3</sup>.

(2) *Sorting and Weighing of Edible Herbage: Percentage Productiveness.* This method, although tedious, undoubtedly gives the best index of the nature of a field from an economic point of view. Two methods of sample-taking have been adopted: (a) Samples of grass (hay) are taken off the swards on the day of cutting, and by a process of mixing and discarding these are worked down to a convenient bulk, sorted and weighed; (b) numerous samples are taken by cutting the produce from within a mesh 6" × 6", and then sorting and weighing as before. This method has proved to be very satisfactory and expeditious, for by having a series of paper bags for the several species a large amount of preliminary sorting can be done upon the field during the process of sample-taking. The produce is kept upon moist blotters

<sup>1</sup> Stapledon, R. G., "Notes on the Weed Flora of some Arable land." *Roy. Agric. College, Cirencester, Scientific Bulletin*, No. 11. for 1910.

<sup>2</sup> This method would seem to be generally applicable to Survey work where large areas are involved.

<sup>3</sup> Armstrong, S. F., "The Botanical and Chemical Composition of the Herbage of Pastures and Meadows." *Journ. of Agric. Science*, vol. 11. Pt 3.

in the laboratory during final sorting, and is therefore weighed as green grass in a condition as nearly as possible similar to that in which it comes off the field.

Results given in terms of percentage frequency cannot, of course, be compared directly to those given by percentage productiveness. It will, however, be shown on some future occasion that the relationship obtaining between them throws considerable light on the relative absolute productiveness of the various species which constitute herbage when growing under different conditions.

## RESULTS.

The results are best shown by considering those obtained by each method of analysis in turn. Discussion of their bearing on drought resistance is postponed, as far as possible, to the general conclusions at the end of the paper. Points of general interest are touched upon as they arise.

### I. PERCENTAGE FREQUENCY.

(a) *Permanent Grass of over seventeen years standing.* (See Tables I and II.)

*Field A.* It is unfortunate that this field was not analysed by the above method in 1910 or during 1911 before the drought commenced. The specific frequency was however obtained in 1910. It is shown in the Table, and will be seen to be very different to that obtaining after the drought had taken full effect. The preponderance of Dutch clover was very marked during 1910, and the field then showed a closely compacted herbage; Armstrong's figures show that when Dutch clover is abundant the number of plants to the acre is very considerable, aggregating from 17 to 20 millions and upwards. As the April analyses give close on 10 millions of plants to the acre and the herbage was sparse and thin and nothing like up to par, it may be safely asserted that the clover prior to the drought would have approximated to 12 millions per acre. The dates of the analyses, viz. October 20th, 1911, February 20th and April 20th, 1912, show the condition of the field (1) right at the end of the drought before any appreciable recuperation had begun; (2) four months later when, under the ameliorating influence of late autumn and early winter rain very considerable improvement in the herbage was manifest; and (3) when the progress of recovery had continued for two more months. The

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figures therefore depict the progress of recovery and not of destruction. The results, given in tabular form, are self-evident and do not call for comment.

TABLE I.

## *Progressive changes in the Botanical Composition of the Herbage on Field A.*

Soil Depth 6"—8" Calcium carbonate 12·7%	Relative frequency	Thousands of plants per acre	Per-centage	Thousands of plants per acre	Per-centage	Thousands of plants per acre	Per-centage	Relative frequency
Dates .....	July 1910	Oct. 20, 1911		Feb. 20, 1912		April 20, 1912		1912
<i>Lolium perenne</i> .....	3	488	48·0	1587	23·1	1981	20·0	1
<i>Poa pratensis</i> .....	6					1742	17·6	2
<i>Poa trivialis</i> .....	4	2	trace	1218	17·7	696	7·0	5
<i>Poa annua</i> .....	—					479	4·8	6
<i>Avena flavescens</i> .....	2	9	·9	171	2·4	936	9·5	3
<i>Cynosurus cristatus</i> .....	1	72	7·1	502	7·3	384	3·7	7
<i>Dactylis glomerata</i> .....	5	59	5·8	196	2·8	195	2·0	8
<i>Phleum pratense</i> .....	trace	—		76	1·1	21	·2	trace
<i>Agrostis stolonifera</i> .....	—	21	2·1	—	—	—	—	trace
<i>Agropyrum repens</i> (g) ..	—	—	—	83	1·2	108	1·1	trace
Total Graminene .....		646	63·9	3833	55·6	6542	65·9	
<i>Trifolium repens</i> .....	1	72	6·9*	435	6·3*	765	8·2*	4
<i>Medicago lupulina</i> }	traces	—	—	—	—	—	—	—
<i>Trifolium minus</i> }		—	—	—	—	—	—	—
Total Leguminosae .....		72	6·9	435	6·3	765	8·2	
<i>Ranunculus bulbosus</i> (g) ..	2	89	8·8	1192	17·3	1720	17·4	2
Other weeds .....	7	195	19·4	1403	20·8	869	8·5	3
Total weeds .....		284	28·2	2595	38·1	2589	25·9	
Totals in millions of plants per acre .....	say 17 to 20	1·0		6·8		9·8		

By February and April the following weeds were also present: *Cerastium triviale* (g), *Stellaria media*, *Bellis perennis* (g), *Cirsium arvense*, *Taraxacum officinale*, *Alchemilla arvensis* (g), *Geranium molle*, *Plantago lanceolata*, *Achillea Millefolium* (g), *Fumaria officinalis*, *Galium Aparine*, *Veronica arvensis*, *V. agrestis*, *V. Buxbaumii*, *V. hederifolia*, *Capsella Bursa-pastoris*, *Euphorbia Helioscopia*.

\* Corresponds to about 2·3 %, 2·1 % and 2·7 % of edible herbage respectively. Plants marked (g) are gregarious and occur in considerable clumps together.

It should be pointed out that owing to the deplorable condition of the field, large bare patches (as much as 3' to 4' by 2' or 3') frequently occurring, sample-taking was rendered most difficult, therefore the

figures must be considered as showing tendencies only, and not as an accurate census of the field. Species marked (*g*) in the table had colonized the barren patches to a greater or less extent, notably by February and April. It was found impossible to differentiate accurately between the several poas till April 20th, when the final relationship between them is shown.

*Several Fields.* A number of fields were analysed by this method during April; the results obtained show their condition after recuperation has been carried a long way and afford a useful comparison to the end stage of field A. The chief features are briefly presented in the subjoined Table (p 136).

In forming a proper estimate of the results it should be borne in mind that although the number of plants to the acre affords much information as to the effect of the drought, the fields cannot be indiscriminately compared with one another, on the strength of one analysis only, since every pasture type tends to have an optimum number of plants to the acre, which optima will vary very much for the several types. The physical condition of the pastures, when analysed in April, however, suggested that the aggregates given are far below the optima for fields F, A, and C, decidedly below it for E (*b*) and B, slightly below for D, and hardly at all for G and H.

It will be convenient here to mention the chief points brought out by the results:

(1) Other things being equal, the thinner the soil the greater the injury.

(2) Other things being equal, a crop of hay, and especially a good crop, would seem to have lessened even the final injury.

(3) Fields that have suffered most are seen to carry the largest percentage of weeds—more weeds occur on the grazed than on the hayed fields.

(4) Apart from the bearing of the figures on drought resistance it is seen that if the results are compared with those presented by Armstrong no very definite relation exists between the optimum number of plants to the acre and the merit of an old pasture. Probably however the optimum for the best old pastures would be found generally to fall between 19 to 23 millions of plants to the acre. Aggregates either much below or much above are only compatible with pastures of inferior type.

TABLE II. Comparison of eight fields to show the relationship between soil-depth and treatment on the one hand, and number of plants to the acre and chief contributing species on the other. April 20, 1912.

Fields	Soil depth	Extent of injury due to the drought	Hay or grazed	Thousands of plants per acre			Total in millions per acre	Dominant species Percentage frequency
				Gramineae	Leguminosae	Weeds		
F	3"—5"	very great	barren rabbit warren	1-651 (69.4)*	29 (1.2)*	693 (29.4)*	3.3	<i>Agrostis vulgaris</i> ... 37.2 <i>Festuca ovina</i> ... 10.5 <i>Poa pratensis</i> ... 15.3 <i>Dactylis glomerata</i> ... 4.4 <i>Cynosurus cristatus</i> ... 4.2 Perennial weeds... 19.7 Annual weeds... 9.7
A	6"—8"	very great	grazed	6-542 (65.9)	765 (8.2)	2 58.1 (25.9)	9.3	see Table I
E (b)	12"—16"	considerable	grazed	8 973 (61.7)	2-766 (19.2)	2 765 (19.1)	14.5	<i>Festuca ovina</i> ... 26.3 <i>Lolium perenne</i> ... 20.7 <i>Trifolium repens</i> ... 18.6
G	36"—40" (alluvium on gravel)	hardly appreciable	grazed	12-675 (74.4)	2-047 (12.1)	2-265 (13.5)	16.9	<i>Festuca ovina</i> ... 21.8 <i>Agrostis stolonifera</i> ... 28.8 <i>Avena pubescens</i> ... 6.8 <i>Trifolium repens</i> ... 9.0
C	8"—10" (sub-soil clay)	very great	Hay (5 cwt. to acre)	6-864 (59.0)	3-396 (28.9)	1-433 (12.1)	11.6	<i>Festuca ovina</i> ... 14.9 <i>Agrostis stolonifera</i> ... 12.8 <i>Festuca elatior</i> ... 6.5 <i>Trifolium repens</i> ... 28.8
B	8"—10"	slight in the hay, more considerable on aftermath	Hay (25 cwt. to acre)	13-307 (89.4)	740 (5.0)	849 (5.6)	14.3	<i>Bromus erectus</i> ... 29.3 <i>Festuca durtuscula</i> ... 36.8 <i>Dactylis glomerata</i> ... 3.2
D	12"—18"	ditto	Hay (20 cwt. to acre)	15-638 (82.4)	2 025 (10.7)	1-263 (6.9)	13.9	<i>Lolium perenne</i> ... 25.6 <i>Poa trivialis</i> ... 13.1 <i>Avena flavescens</i> ... 13.0 <i>Trifolium repens</i> ... 10.4
H	36"—38" (clay)	hardly appreciable	Hay	19-233 (76.0)	4-464 (16.8)	1-964 (7.2)	26.6	<i>Festuca ovina</i> ... 24.6 <i>Poa trivialis</i> ... 14.3 <i>Lolium perenne</i> ... 10.6 <i>Trifolium repens</i> ... 13.2

\* The figures in brackets show percentages.

(b) *Leys: 2—4 years' duration.* (See Table III.)

The results given in Table III show both the mixtures used and the botanical composition of two leys which are compared to a natural tumble-down pasture of similar age—thus giving accurate information as to the relative success of the sown and endemic species.

The information derived from these figures is drawn upon in the general conclusions.

## II. SPECIFIC FREQUENCY.

### *Barren Rabbit Warren at Colesborne.*

The conditions that obtain here are eminently critical, every species that occurs on the area being *prima facie* a drought resister. Consequently it is of importance to learn not merely what species but what general growth forms have succeeded best. Analyses by the species method have been made over four characteristic areas. It is found that the chief contributing species amount to 76 in number. The percentage frequency is given for one area (see Field F, Table II), which shows: (1) the herbage to be excessively sparse, only giving 2·5 millions plants to the acre, and (2) the flora to be markedly non-gramineous, about 30% being a miscellaneous assemblage of dicotyledonous with a few monocotyledonous plants. With a view to gauging the success of the various growth forms the following classification based upon the species actually encountered on the fields analysed is of interest.

### *Review of the Morphological characters of the Flora.*

1. Annuals	...	...	...	...	about 20 % of the total species
2. Biennials and Perennials:					
(a) With deep growing, generally thickened root systems. Vegetative organs tufted or erect	..	...	..	...	„ 25 „ „ „
(b) Deep rooted and with thickened creeping stems or rootstocks	...	...	...	...	„ 16 „ „ „
(c) Root depth variable: runners, stolons, rootstocks or offsets not much thickened or if so fleshy	...	...	...	...	„ 27 „ „ „
(d) Not very deep rooted: considerably thickened runners, stolons or rootstocks	...	...	...	...	„ 9 „ „ „
(e) "Bulbous" or tuberous plants	...	...	...	...	„ 2 „ „ „
(f) Shallow rooted non-creeping non-thickened hypogeal organs	...	...	...	...	„ 1 „ „ „



TABLE III.

Shows relation between Seed-Mixtures used and the Botanical Composition of the herbage of two Leys. The composition of a field that has tumbled-down naturally is given for comparison.

Situation	Leys at Cirencester				Tumble-down at Colesborne
Soil depth	Sown 1910		Sown 1908		After Wheat 1908
Cirencester 6"—9" Colesborne 4"—7"	Mixture in lbs. per acre	Percentage April, 1912	Mixture in lbs. per acre	Percentage April, 1912	Percentage July, 1911
<i>Lolium perenne</i> .....	12½	38·6	5½	31·1	—
<i>Lolium italicum</i> .....	10½		3½		—
<i>Avena elatior</i> .....	1	·5	1	·2	—
<i>Avena flavescens</i> .....	—	—	—	—	3·2
<i>Dactylis glomerata</i> .....	1	9·2	4	11·5	—
<i>Phleum pratense</i> .....	2	14·3	2	18·9	—
<i>Poa pratensis</i> .....	1	·9	—	2·1	20·0
<i>Poa trivialis</i> .....	—	—	—	—	—
<i>Alopecurus pratensis</i> .....	2	—	—	—	—
<i>Festuca ovina</i> (vars.) .....	4	3·2	2	3·1	68·8*
<i>Festuca elatior</i> .....	½	—	3	—	—
<i>Festuca pratensis</i> .....	2	—	4	·3	—
<i>Cynosurus cristatus</i> .....	—	—	—	·8	—
<i>Bromus mollis</i> (and <i>B. sterilis</i> ) .....	—	·1	—	4·6	1·0
<i>Agrostis stolonifera</i> .....	—	—	—	·3	—
<i>Agropyrum repens</i> .....	—	—	—	·3	—
Total Gramineae .....	—	66·8	—	73·2	93·0
<i>Trifolium repens</i> .....	1½	9·5	2½	1·5	1·1
<i>Trifolium pratense</i> (and vars.) .....	8	4·0	3	·3	—
<i>Trifolium hybridum</i> .....	2	3·0	1	·2	—
<i>Trifolium minus</i> .....	½	1·1	—	—	—
<i>Medicago sativa</i> .....	1	·3	—	—	—
<i>Medicago lupulina</i> .....	1	—	—	—	—
<i>Lathyrus pratensis</i> .....	—	—	—	—	1·7
Total Leguminosae .....	—	17·9	—	2·0	2·8
Perennial weeds .....	—	8·2	—	10·4½	4·2
Annual weeds .....	—	7·1	—	14·4½	
Total weeds .....	—	15·3	—	24·8	4·2
Totals in millions of plants per acre .....	—	1·8	—	1·5	—

\* = *Festuca duriuscula*.

By way of giving a general idea of the nature of the flora and emphasizing the above classification, examples of plants belonging to each class are given below. The figure against each species represents its specific frequency (i.e. the number of times it occurred per 100 readings—averaged from four areas).

*Details of Flora classed as above showing Specific Frequency.*

(g) Gregarious in groups together, (s) solitary.

1. *Linum catharticum* 29, *Alchemilla arvensis* 8, *Myosotis scorpioides* 3, *Arenaria serpyllifolia* 3, *Festuca sciuroides* 4, *Draba verna* 6, *Sonchus asper* 2, *Euphrasia officinalis* 5.

2. (a) *Dactylis glomerata* 4, *Taraxacum officinale* 14, *Poterium sanguisorba* 50, *Silene inflata* 3, *Plantago lanceolata* 3, *Geranium dissectum* 1.

(b) *Lotus corniculatus* 18, *Galium verum* 5, *Carex glauca* 47, *Brachypodium pinnatum* (g), *Achillea Millefolium* (g).

(c) *Carduus arvensis* 24, *Lathyrus pratensis* 8, *Agrostis vulgaris* 40, *Festuca ovina* 60, *Poa pratensis* 10, *Avena flavescens* 4, *Briza media* 6, *Prunella vulgaris* 16, *Ranunculus repens* 7, *Veronica Chamaedrys* 15.

(d) *Viola hirta* (vars. and hybrids 17), *Carduus acaule* 5, *Senecio Jacobaea* 30.

(e) *Ranunculus bulbosus* (s), *Orchis apifera* (s).

(f) *Anthoxanthum odoratum* (s).

The above classification can only be approximate, the distinctions being arbitrary; but it certainly shows:

(1) That drought resistance is not correlated with any one set of morphological characters.

(2) That the creeping habit associated with thickened underground vertical or horizontal organs is as efficacious, or even more so, on these thin soils than a penetrating root system.

(3) That certain annuals are very adaptable to conditions of drought: annual weeds constitute 9·7% of the total herbage (Table II). They are mostly early flowering ephemerals<sup>1</sup>.

It affords hints as to morphological types suitable for cultivating on barren land as a preliminary to establishing a remunerative herbage.

<sup>1</sup> Abundance of annuals is a noteworthy feature on most heath grass land. See Tansley, *Types of British Vegetation*, pp. 94—97. Camb. Univ. Press.

## III. PERCENTAGE PRODUCTIVENESS.

(See Table IV.)

This comprises a series of analyses taken before the commencement of, during, and after the termination of the drought. The full results are set out in the Table (Table IV) herewith.

In order properly to substantiate the final conclusions something must here be said about the individual fields.

*Field B.* *Bromus erectus* is seen to have been more abundant in 1910 than in 1911. The reduction was due to heavy grazing and subsequently harrowing the aftermath in 1910. The effect of the drought has been to bring back the brome to its previous position despite endeavours to keep it in check. The frequency of *Festuca duriuscula* is greater than that of the brome, viz. 36·8:29·3 (see Table II), which shows how very productive the brome grass is on these soils. A comparison of the absolute productiveness of brome, cocksfoot and rye grass was made (by weighing the produce of 100 plants of each) with the result:

Erect brome : cocksfoot : perennial rye grass :: 140 : 100 : 57.

*Field C.* Shows that even on thin soils, if somewhat retentive of moisture, Dutch clover is not completely killed; here, although giving only 3% of the hay, it had advanced to about 12% of the edible herbage by the Spring. (See Table II, showing 28% frequency = about 12% productiveness.)

*Field E.* (a) is the only field which shows an increase of clover from June to October. This must be due to the fact that a supply of moisture is fortuitously maintained here which kept up a good supply of coarse gramineous herbage, with the result that both shade and moisture were provided for the clover.

*Field I.* The flora is similar to that of other water meadows, the chief feature being excess of Yorkshire fog, rye grass and the creeping buttercup, accompanied by a paucity of clovers<sup>1</sup>. In the present connection it is instructive to note the good yields provided by soft brome, tall oat, crested dog's-tail and meadow barley grass; all grasses met with

<sup>1</sup> E.g. at Chester. See also Dr Fream, *Journal of Linnean Soc. (Bot.)*, vol. xxiv. 1888. The association of Yorkshire fog and creeping buttercup with pastures on deep retentive soils is a common occurrence and detracts from the merit of acres of grass land in the West of England.

TABLE IV. Botanical composition of the Herbage of Five Fields. (Percentage productiveness.)

Fields	B				C	D	E (a)		I
	Hay (25 cwt. to acre)				Hay (50wt. to acre)	Hay (20 cwt. to acre)	Grazed (coarse herbage)	Hay (2 ton to acre)	
Water content	Dry				Dry	Slightly moist	Decidedly moist	Irrigated water-meadow	
Soil depth	6"—8"—10"				8"—12"	12"—18"	12"—13"—22"	—	
Dates	June 17 1910	May 20 1911	June 3rd 1911	Oct. 31 1911	April 20 1912	June 6 1911	June 10 1911	June 9 1911	June 10 1911
<i>Lolium perenne</i> .....	2.2	1.4	6.9	6.0	4.0	19.7	35.0	17.0	30.2
<i>Dactylis glomerata</i> .....	8.5	6.7	10.2	21.5	9.9	21.9	24.3	21.3	8.0
<i>Alpecurus pratensis</i> .....	—	—	—	—	—	—	2.9	32.3	3.2
<i>Avena flavescens</i> .....	1.3	1.0	1.8	1.0	.3	1.8	10.0	.5	2.0
<i>Avena elatior</i> .....	—	—	—	—	—	—	12.6	—	5.3
<i>Avena pubescens</i> .....	.2	.3	.1	—	.4	.9	—	—	—
<i>Poa trivialis</i> .....	1.7	4.4	2.8	3.5	1.0	1.3	5.1	2.8	6.3
<i>Poa pratensis</i> .....	8.1	13.8	14.8	17.0	14.9	1.6	1.8	6.1	2.2
<i>Festuca duriuscula</i> .....	—	—	—	—	—	21.1	22.0	8.4	—
<i>Festuca elatior</i> .....	.6	.8	.4	—	.1	.6	1.6	29.3	22.0
<i>Holcus lanatus</i> .....	.4	—	.5	.5	.2	1.4	1.8	1.9	8.5
<i>Cynosurus cristatus</i> .....	—	—	.9	—	—	—	—	.4	.6
<i>Anthoxanthum odoratum</i> .....	—	—	—	—	1.6	—	2.6	—	4.6
<i>Bromus mollis</i> .....	.3	6.7	1.8	—	58.1	6.6	—	—	4.5
<i>Bromus erectus</i> .....	62.0	44.8	45.3	41.0	—	—	—	—	2.0
<i>Hordeum pratense</i> .....	—	—	—	1.0	.2	—	—	.4	—
<i>Agrostis stolonifera</i> .....	—	.1	—	—	1.7	—	—	—	—
<i>Aira caespitosa</i> .....	—	—	—	—	—	—	—	—	2.7
Other grasses .....	—	—	—	—	—	—	—	—	—
Total Gramineae .....	85.3	80.0	85.5	91.5	93.3	79.9	85.8	82.9	97.1
<i>Trifolium repens</i> .....	9.2	7.7	5.7	2.0	1.5	3.0	6.3	6.3	.4
<i>Trifolium pratense</i> .....	—	1.0	.6	.2	—	—	5.2	2.8	—
<i>Lathyrus pratensis</i> .....	—	1.4	.3	.8	.7	.3	—	—	—
Total Leguminosae .....	9.2	10.1	6.6	3.0	2.2	3.3	11.5	9.1	.4
<i>Ranunculus bulbosus</i> .....	—	—	—	—	—	—	—	—	—
<i>Ranunculus repens</i> .....	5.5	8.8	7.9	3.0	4.2	12.8	2.0	8.0	5.1
Other weeds .....	—	1.1	—	2.5	.3	4.0	.7	—	—
Total weeds .....	5.5	9.9	7.9	5.5	4.5	16.8	2.7	8.0	2.5

+ the species thus marked under field D have together contributed 23 % to the herbage.

in moderate quantity on dry situations. Although not having succeeded very well (except *B. mollis*) under the exceptional conditions of the summer of 1911, they are usually good drought-resisters. This shows that ability to resist drought is not incompatible with a capacity to succeed under conditions the direct antithesis to those obtaining on dry thin soils. Again, despite the fact that Yorkshire fog, creeping buttercup and marsh thistle attain to great luxuriance under humid conditions, it is remarkable that not inconsiderable quantities of all of them (though stunted specimens) occur on the barren pastures at Colesborne. It is evident therefore that drought resistance is not of necessity an absolute character, depending on morphological adaptation, but in many cases is but an outcome of the plant's inherent vitality.

#### GENERAL CONCLUSIONS.

The foregoing analyses, in conjunction with a number of close observations made on other fields in the immediate neighbourhood, justify the following conclusions:

##### (a) *General.*

(1) The critical soil depth seems to have been about 9", pastures on soils of less depth having suffered to a far greater extent than those on soils of 10" and upwards. Soils of 14" and 18" have produced, in many cases, very good yields of hay of good quality.

(2) Pastures on soils of about the critical depth have suffered more when closely grazed than when a crop of hay has been taken. In the case of one field analysed (A, see Table I) the reduction in the herbage was remarkable, for by October 22nd the aggregate number of plants on the field was but little over one million, having fallen from something between 16 and 20 millions as a direct result of the drought. Other analyses have shown a very different relationship between the component species on a field in October to what obtained before the drought began.

(3) The fields that suffered the greatest damage were those which in 1910 carried large quantities of Dutch clover. This plant died out in a wholesale manner, large bare patches being left on which the dead runners of the clover could be seen. On these patches the finer grasses, such as rough-stalked meadow grass, crested dog's-tail and golden oat grass had almost failed. (Fields A and C are examples.)

(4) As the season advanced the bare patches began to be colonized

by seedlings of the bulbous buttercup (*Ranunculus bulbosus*), daisy, and mouse-eared chickweed (*Cerastium vulgatum*), and some by runners of the persistent yarrow. On some fields, especially long leys, large amounts of bindweed (*Convolvulus arvensis*) and dandelion (15% on one field) were in evidence. Arable land weeds also began to put in an appearance.

(5) The extent of recuperation by the middle of February was surprising. On Field A (see Table I) the aggregate flora had increased since the middle of October from about one million to over six millions plants to the acre. By the middle of April the recovery had been carried a considerable stage further, the total number of plants to the acre then being 9·8 millions. The manner of recuperation was very different for the different fields, and would seem to have depended chiefly on the potential vitality of the stoloniferous plants (e.g. *Trifolium repens* and *Poa pratensis*).

(6) Recovery on the bare patches was, however, not satisfactory, the clovers and valuable grasses having done practically nothing to reclaim them. Instead they were colonized by little closed associations of daisy, buttercup, or mouse-eared chickweed seedlings. Some arable land weeds had also established themselves in force, chief of these being the little parsley piert (*Alchemilla arvensis*), a weed normally only found in small quantity, if at all, in pastures of this district, but one very common on sainfoin leys and arable fields generally: here it had colonized areas of yards together. Speedwells, chickweed, fumitory, sun spurge, cleavers, and the like, were also much in evidence. Couch (*Agropyrum repens*) had established itself on many fields, generally taking a part in the colonization of the bare patches. The same is true of *Poa annua*. Moss, which was in evidence during the summer, had increased extensively by the spring on grazed fields where it occupied positions not taken up by Angiospermic species on the hard bare patches.

(7) Most of the grasses (notably *Lolium perenne*) ripened and seeded early. Much ripe seed was carried with the hay and so could not benefit the parent field; but when the hay was fed to stock on grass land the seed was shed, as was rendered apparent in the autumn by the presence of brilliant green patches of rapidly growing seedlings where the pens had previously stood.

(8) The severe frost during the early part of February does not seem to have retarded the rapid recovery of the pastures.

*(b) The several Species.*

Clovers. In general they fell to a very small bulk during the summer—on grazed fields on thin soils only giving about 2% of the edible herbage (footnote Table I). Under hay their productiveness was directly proportionate to the yield. On all the fields, except one, they attained to their maximum relative productiveness towards the middle of May. Before the hay was cut the productiveness decreased<sup>1</sup>, and showed a progressive decline in the aftermath till October. On Field B the clover fell by 7% relative to other plants between May 20th and October 31st.

Dutch Clover (*Trifolium repens*) did not begin to recuperate till after the middle of February. On the thinnest soils, especially where no hay was taken, the plant was killed to such an extent that recovery to date has been very slight and it may never regain its former position. On fields of better soil-depth (10" and over) or even on thin soils more retentive of moisture (e.g. Field C, Tables II and IV) it has evinced great powers of recuperation, in one instance having come on fourfold since the summer. The following facts point to the conclusion that the continuous scorch of the sun had as deleterious an effect on the growth of this plant, especially with regard to its productiveness through the summer, as had the actual drought, thus:

- (1) its almost total failure on some closely-grazed fields;
- (2) its much greater failure in the hay crop on meadows which produced a very poor, than those which produced a very good, yield of hay; and
- (3) its progressive falling off in bulk on the aftermath, the only field where it maintained an average yield till October being one where not only was a good supply of water available, but the herbage was coarse and shade-giving. (See Table IV E(a).)

Red Clover (*Trifolium pratense*). The deep-rooted varieties have proved moderately resistant on two and four-year-old leys. The greater failure of even the deep-rooted varieties than of Italian rye grass on short leys is probably to be attributed to the sun: the more so as the clovers have failed more in the aftermath than in the hay. Many leys are now to be seen consisting almost entirely of rye grass. The greater failure of the clovers in the leys may, however, be in part due to the

<sup>1</sup> On Field B the standing crop was estimated on May 20th as equal to 25 cwt. of hay, it did not increase at all by June 3rd when it was cut. The coarser grasses came on but the clovers had begun to decline.

baneful effect, claimed by many authorities, of rye grass upon clover, an effect more likely to be apparent during a critical than a normal season.

Alsike (*Trifolium hybridum*). On leys of two and four years duration this contributed appreciably to the bulk of the herbage and would thus seem to be a useful plant for leys even on these thin soils.

Yellow Suckling Clover (*Trifolium minus*) and Black Medick (*Medicago lupulina*). Both of these plants were less frequent than usual on fields that normally carry them. On leys the medick sown in 1910 was not present at all in samples taken in April 1912.

Bird's-foot Trefoil (*Lotus corniculatus*) and Meadow Vetchling (*Lathyrus pratensis*) showed themselves very resistant. On barren pastures at high elevations and on shallow-soiled fields they came up in great quantity in the spring of 1912. *Lathyrus* would seem to have bulked more heavily in the 1910 hay crop than in that of 1911—but it was more abundant in the 1911 aftermath than usual. Speaking generally, it is not a good aftermath plant.

#### *Grasses.*

Perennial Rye Grass has everywhere shown itself wonderfully resistant and persistent. All the analyses show a progressive relative increase from May to October, when it reached its maximum relative productiveness, thence falling in position as less resistant species began to recover and the quantity of the creeping resisters increased. Compared with its productiveness under ideal circumstances it would seem to have lost in absolute productiveness (on these soils this season) as 70:47<sup>1</sup>. The ubiquitous distribution of this grass is noteworthy. It was present on nearly all the pastures investigated in quantities from 6% to 30%. It does not however seem to have established itself to any extent on the barren uplands at Colesborne, but even there on fields that have been put down to grass it ranks about fifth in order of frequency<sup>2</sup>.

On many fields it showed a tendency to emit short stolons, bearing little tufted plantlets at the nodes. The inflorescence frequently approximated to the type *racemosum*<sup>3</sup>.

<sup>1</sup> Ascertained by weighing 100 plants of each, setting large against large and small against small and so forth till the full number is obtained. The ratio is given on the false assumption that cocksfoot is here fully productive, so that the rye grass figure should be less in proportion to the depressed productiveness of cocksfoot.

<sup>2</sup> Nor has it established itself much on tumble-down pastures. It may perhaps not be endemic on the Cotswolds.

<sup>3</sup> I am indebted to Mr S. F. Armstrong for identification of typical specimens collected in 1910.



Cocksfoot (*Dactylis glomerata*). Like other resistant species it came on progressively from May to October, then falling off through the spring. It now occupies practically the same position as it did before the drought. This shows that the individual plants bulked well through the drought: but by virtue of its caespitose habit it has not been able to win ground at the expense of the less resistant species.

Hard Fescue (*Festuca duriuscula*) on fields where it may be regarded as endemic has proved very resistant and surprisingly luxuriant, having in one instance contributed as much as 14% to the hay. On such situations it has behaved in a manner similar to cocksfoot, except that in virtue of a somewhat creeping habit it has been able to occupy more ground. Consequently, although falling slightly since October, it now stands considerably higher than it did in 1910 (as 14.9%: 8.1%).

On leys that have come under observation where this and other varieties of sheep fescue have been sown, they have not proved successful, nor have they got hold of the ground even in proportion to the amount of seed sown (Table III).

On tumble-down leys of equal age and on fields apparently similar the endemic variety has, however, established itself naturally in great quantity (see Table III).

From this it would seem:

- (1) That varieties exotic to the neighbourhood are of no value here.
- (2) That the endemic species is either very capricious in establishing itself on one field and not on another, or that sowing down with a full mixture serves to completely prevent it coming in naturally on a field.

In 17 years on Field C (sown with a full mixture) it only contributed 1.6% to the herbage as opposed to 68% in four years on the tumble-down.

- (3) It is probable that if large amounts of seed from the endemic variety could be collected, results as satisfactory as those recently obtained by the use of wild Dutch clover might be forthcoming.

Erect Brome Grass (*Bromus erectus*) has given a great bulk of herbage all through the drought, and since it has a decidedly creeping habit it has increased its hold on fields since 1910. This plant is greatly reduced, and may ultimately be almost entirely eliminated by constant and close grazing. On one field where constant grazing had reduced it to an unobservable amount it may now be seen in little tufts scattered

all about the field, thus affording singular testimony as to its capabilities as a drought resister.

Where hay is required on the thinner soils the presence of this grass is a very important factor. When abundantly present good yields of hay (dominated by this grass) have been harvested. Compare, for instance, 25 cwt. from Field B with 45% erect brome, to 5 cwt. from Field C with 6.6% of that grass. The soils of both fields are below the critical point. Erect brome also, by the shade it offered to the clovers, contributed to their better yield on the former field. It was noted also that the soils cracked less when protected by the brome, and carried a smaller amount of miscellaneous herbage. It must be pointed out, however, that this dominance of a relatively coarse bulky grass tends actually to depress the absolute productiveness of most of the other component species, not even excepting cocksfoot. Like hard fescue it is endemic on the Cotswolds; it does not, however, seem to come in so rapidly on tumble-downs as the fescue, but when fully established is equally dominant and far more productive. Two distinct varieties have been observed, the normal with rather narrow involute leaves, and one with much broader leaves and longer marginal hairs placed further apart. It is everywhere attacked to a variable degree by *Ustilago hypodytes*, the diseased plants never flowering and assuming a characteristic stunted very distichous form. In 1912 the attack was as bad as ever. As some difference was seen on the different manurial plots, which have been in continuance for over 23 years on Field B, analyses were made, some of which are here given:

Kainite alone ... ..	13%	smutted
Kainite and superphosphate ... ..	10%	"
Unmanured ... ..	10%	"
Kainite, superphosphate, and sodium nitrate	9%	"
Sodium nitrate ... ..	no smut.	

Soft Brome (*Bromus mollis*) was very abundant on many hay fields, but seeded and died down very early, so that it did not itself bulk largely in the hay crop (except in exceptionally wet fields or on irrigated meadows, when the abundance of moisture lengthened its period of vegetative growth), but it balked the growth of better and later grasses, and was, therefore, in many cases a factor in reduced hay yield. As a result of early and prolific seeding in 1911 it is now to be seen coming up in great quantity even on fields where previously it was present in small quantities only.

Tall Fescue (*Festuca elatior*), on the thin soil of Field C, withstood the drought well, giving 21% of the meagre hay crop harvested there. On the leys (Table III) it has not shown to advantage, but this was apparently due to a failure on the part of the seeds to establish themselves in the first instance.

Meadow Fescue (*Festuca pratensis*) is not suited to these thin dry soils and has nowhere shown to advantage. It has completely died out in 17 years on Field C, and only contributes a trace to the herbage on short leys.

Timothy (*Phleum pratense*) on leys of two and four years duration has proved itself surprisingly resistant. It gave a percentage frequency only second to rye grass (Table III), nor was the produce very poor or the plants strikingly "bulbous." The absolute productiveness per unit plant<sup>1</sup> compared to that of cocksfoot was as 57.9:100—the figure for the grass growing under ideal conditions being 75:100. Consequently timothy would appear to be of value as a short ley grass even on moderately thin soils (8" and upwards).

Meadow Foxtail (*Alopecurus pratensis*) on the thin soils does not establish itself, and is of no use as an ingredient for even short leys. On deeper soils (12" and upwards) it contributes well to the herbage of older fields. On Field D it is abundant, but was reduced to 2.9% of the hay during the summer, but the drought there did it no lasting harm, as by the spring it was very luxuriant and gave 10% of the ground herbage.

Tall Oat (*Arrhenatherum avenaceum*), although having the reputation of a drought-resister, does not contribute largely to the herbage of this district, and when used on short leys does not establish itself satisfactorily. As an endemic grass it occurs in quantity at the bottoms of dry hedges, and in young plantations it colonizes large patches under the slight shade of larch and other trees even when the conditions are very dry (soil 5"—9"). This suggests that the grass is a dry-place shade-grass, and its failure on poor pastures is due more to the lack of shade than to simply arid conditions.

Yorkshire Fog (*Holcus lanatus*), with regard to productiveness, has suffered considerably. Large amounts occurred only where moisture was maintained by fortuitous circumstances (Fields E (a) and I, Table IV), but even when so placed it decreased in quantity as the season advanced. As small stunted specimens it withstood the drought even on the barren pastures at Colesborne.

<sup>1</sup> See footnote under Rye Grass.

Crested Dog's-tail (*Cynosurus cristatus*) undoubtedly suffered very much, and has not maintained its reputation as a drought-resister. It has bulked best on the wetter, deeper-soiled fields. On Field A it dropped from a dominant position in 1910 to fifth position in 1912. It only contributed 2% of the herbage on barren pastures at Colesborne and 4% on the deeper-soiled closely grazed E (b). Careful search was made for the grass on another grazed field where it was formerly very abundant, but the number of plantlets found was surprisingly few. The above facts suggest that the excess of sun has played an important part in its reduction.

Golden Oat Grass (*Avena flavescens*) fell very low on the grazed fields below the critical soil depth, but has seldom been completely destroyed, and has recovered remarkably since the autumn. It maintained an average yield on some of the deeper-soiled meadows. It is indigenous to the neighbourhood and occurs to some extent on the barren pastures at Colesborne, and has come in naturally, both on the four-year tumble-down and on Field C.

Fiorin (*Agrostis stolonifera* and *A. vulgaris*) on many of the shallower-soiled fields certainly increased considerably above the normal as the result of the drought. *A. vulgaris* is seen to be the most resistant grass on the barren pastures at Colesborne, but it is very unproductive there.

The Poas have behaved differently on different fields, but dropped much in productiveness through the summer.

Smooth-stalked Meadow Grass (*Poa pratensis*). A very widely distributed endemic grass has come in naturally in good amount on Field C (Table IV), and on four-year tumble-down (20%, Table III). On short leys its abundance does not show any relation to the amount of seed sown, consequently it is doubtful if it is ever worth including in mixtures. It gave a very sparse herbage through the summer, but it was not killed and was the first grass to regain position early in the autumn. On Field A (Table I) it has changed from sixth in order of frequency in 1910 to second in 1912, contributing 17.6% of the plants to the acre, although on October 31st it was discernible only in traces—thus it has won much ground at the expense of less resistant species.

Rough-stalked Meadow Grass (*Poa trivialis*) was reduced to a minimum on the thin-soiled fields and in part killed right out; even on the deepest it was reduced far below par, but on these has done much to regain position during the spring.

*Miscellaneous Plants.*

Bulbous Buttercup (*Ranunculus bulbosus*) has shown great failure in the production of 1912 corms; this has, however, been more than counterbalanced by the numerous seedlings produced.

Yarrow (*Achillea Millefolium*) on the thin soils produced very little herbage through the summer. The stolons were not killed and the plants gained much ground during the spring.

## SUMMARY.

The following brief summary may be given on the aetiology of drought resistance as exemplified by the conditions obtaining in 1911.

From an agricultural point of view the value of a plant as a drought-resister is seen to be measurable by two standards: (1) Its power to give a good yield all through the period of drought; (2) its ability through living through the summer without materially adding bulk to the herbage to recuperate when the conditions become less severe. It has been shown that the phenomenon of drought-resistance is not associated with any one set of morphological characters, but that various growth forms are met with amongst the most successful plants. Further, a number of plants have shown themselves very tolerant, although having no apparent modifications to assist them, in which case there can be no doubt that their power of resistance is a simple outcome of their inherent vitality. Consequently it is perhaps dangerous to assign too great an importance to the possession of apparently useful modifications. A fair correlation is however seen to exist between a plant's manner of resistance and its growth form, as the following classification shows.

*Perennials.*

(1) Plants which give a high absolute productiveness all through the summer:

(a) Spot bound (caespitose and erect) plants which do not gain actual ground on the fields they occupy, i.e. their percentage frequency is not appreciably higher in the spring following the drought than it was in the spring preceding it, e.g. *Dactylis glomerata* (deep root system), *Lolium perenne* (inherent vitality), *Phleum pratense* (two- and four-year leys), *Poterium sanguisorba* and *Onobrychis sativa* (deep root system).

(b) Slightly creeping plants which gain ground on the fields they occupy, e.g. *Bromus erectus* (deep rooted and thickened), *Festuca duriuscula* (thickened), *Festuca elatior* (deep rooted), *Lathyrus pratensis* (thickened offsets).

(c) Creeping plants which gain much ground on the fields they occupy, e.g. *Brachypodium pinnatum*, *Carex glauca* and *Lotus corniculatus* (deep rooted and thickened creeping root systems and stolons).

(2) Plants which give a poor absolute productiveness through the summer, but which, in the main, are not killed : \*

(a) Spot-bound plants which become productive with the advent of better conditions, but cannot win new ground, e.g. *Trifolium pratense* (vars.) and *T. hybridum* (short leys)

(b) Those which evince great recuperative powers, rapidly regaining and often winning position on the ground, e.g. *Poa pratensis* (moderately deep but not much thickened stolons), *Agrostis stolonifera* (shallow stolons), *Avena flavescens* (creeping slightly thickened root system), *Achillea Millefolium* (moderately deep, thickened, creeping stock) and plants of the type *Potentilla anserina* and *Prunella vulgaris* (shallow runners and inherent vitality).

(c) Those which remain small and stunted, peopling adverse habitats to some extent, but never productive, e.g. *Holcus lanatus*, *Anthoxanthum odoratum* and *Cynosurus cristatus* (sometimes due to inherent vitality).

(3) Plants which are not drought resisters, being killed under trying conditions, and which show on practically all situations a depressed productiveness through the summer and recover in the spring in inverse proportion to the severity of the habitat :

(a) When not destroyed regain position in the spring, e.g. *Trifolium repens* (shallow runners) and *Poa trivialis* (shallow decumbent runners), *Alopecurus pratensis* (slightly stoloniferous, vide patches of this grass on deeper-soiled pockets on shallow fields). *Arrhenatherum avenaceum* (creeping root system : gregarious).

(b) Even when not killed unable to regain ground in the spring, e.g. *Festuca pratensis*, *Cynosurus cristatus* (despite deep root system), *Phleum pratense* (except on short leys and pastures where stunted specimens are endemic), *Holcus lanatus* (except on pastures where stunted specimens are endemic).

I am indebted to Mr Russell Swanwick for information with regard to the fields on the College Farm, to Mr Bruce Swanwick and Mr H. Elwes, F.R.S., for kindly allowing me the run of their grass land and for information as to same. My thanks are also due to Mr M. Kershaw for help in preparing the tables and for several chemical analyses, and to Prof. Kinch for valuable advice in the matter of sample-taking and selection of plots.

# THE EFFECT OF PARTIAL STERILISATION OF SOIL ON THE PRODUCTION OF PLANT FOOD.

## PART II. THE LIMITATION OF BACTERIAL NUMBERS IN NORMAL SOILS AND ITS CONSEQUENCES.

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IN our earlier communication<sup>1</sup> we showed that bacteria can no longer be regarded as the only active inhabitants of the soil. We obtained evidence of another group of organisms, detrimental to bacteria, and differing from them by their larger size, slower rate of multiplication under soil conditions, and lower power of resistance to heat and to antiseptics. These are more readily killed than bacteria, and we regarded their suppression as an important factor in determining the increased bacterial activity known to set in after soil has been partially sterilised. Such properties as we were able to ascertain agreed with those of the protozoa, for which we were thus led to look: we found representatives of each of the three groups, ciliates, flagellates and amoebae. We therefore supposed that some of these protozoa constituted the detrimental organisms indicated by our experiments.

Subsequent experiments made by ourselves, by Goodey, Martin, and others, have shown that numerous kinds of protozoa occur in the soil, but they have also revealed a new difficulty, that of ascertaining precisely which kinds are leading their trophic life in the soil and which kinds are present only as cysts. As this problem is not likely to be solved till much more work has been done from the zoological side we decided in the meantime to continue our experiments from our own point of view and to determine the effect on soil fertility of these

<sup>1</sup> This *Journal*, 1909, 8, 111—144.

conflicting groups of organisms. These experiments form the subject of the present paper.

The investigation falls naturally into two parts. In the first instance it is necessary to determine the effect on bacterial numbers of the presence of the detrimental organisms. It follows as a simple deduction from the existence of these organisms that the number of bacteria present per gram of soil at any given time does not depend primarily on the temperature, the water supply or other conditions of the soil, but on the difference in activity of the two groups. Thus a rise of temperature favours not only the bacteria but also the detrimental organisms, and if the latter happen to be favoured more than the former the bacterial numbers will fall. Experiment shows that this deduction is correct. No sort of relationship can be traced between bacterial numbers and temperature, the numbers sometimes rising, sometimes falling, and sometimes being unaffected by rise of temperature (Table II). Increases in the amount of soil moisture may or may not increase the numbers of soil bacteria. These erratic effects are not peculiar to our own soil, but are general and have caused much perplexity and not a little controversy among soil bacteriologists in the past (§ 5). They are entirely explicable on our view that bacterial numbers simply represent the difference in activity of bacteria and the detrimental organisms. Further confirmation is found in the fact that in partially sterilised soils (from which the detrimental organisms are absent) the bacterial numbers increase in a regular manner with rise of temperature and of water content (Figs. 1, 2). Increases in the amount of organic matter in the soil also fail to increase bacterial numbers to a corresponding extent and may indeed, lead to soil "sickness" as shown in previous papers<sup>1</sup>. But when the detrimental organisms are suppressed by partial sterilisation the expected rise in bacterial numbers sets in and the phenomena of "sickness" are not seen.

Similarly any other factor favourable to the growth of living organisms may effect a reduction in bacterial numbers through bringing about a development of the detrimental organisms. *Vice versa*, causes which in themselves are unfavourable to growth may nevertheless lead to increases in bacterial numbers through suppressing the detrimental organisms.

In the second place we have attempted to trace the connection between bacterial numbers and soil productiveness. Other circumstances

<sup>1</sup> This *Journal*, 1912, 5, 27, 86.



being equal, the greater the numbers of bacteria the more rapid is the production of ammonia and of nitrate in the soil. But we find that an accumulation of either of these substances, and especially of ammonia, tends to stop further accumulation, even though the bacterial numbers increase, so that the relationship between ammonia production and bacterial numbers is no longer seen. No sharp relationship can be looked for in any case because our methods of counting bacteria afford only very rough approximations. There is evidence, however, that the fluctuations shown by the gelatine plate methods correctly reflect the fluctuations of the total numbers of decomposition bacteria; when used for this purpose the data are distinctly valuable. It may happen, however, that the productiveness of the soil is limited by some other factor such as temperature, water supply, insufficiency of calcium carbonate, of phosphates, potassium compounds, etc. and that the additional supply of nitrogenous food is therefore ineffective to raise the crop. Increases in bacterial numbers only increase the productiveness of the soil when the conditions are such that the increased numbers can make more ammonia and nitrate, and when no other limiting factor intervenes to prevent this extra nitrogenous plant food from causing more plant growth.

We have also dealt with some of the objections raised against our main conclusions that organisms exist in the soil detrimental to bacteria, and that the bacterial numbers at a given moment are determined by the mutual interaction of these conflicting groups. It has been asserted that no such organisms exist, and in particular that ciliates and amoebae could not lead a trophic life in the soil. Others have supposed that our results are due to an improvement in the bacterial flora brought about by partial sterilisation, either through the suppression of certain forms detrimental to food-making bacteria or through a stimulus resulting from the treatment and transmitted to the descendants of the surviving organisms. No experimental proof is offered in support of these views and we have been able to adduce direct evidence against them. Our results have also been attributed to the presence of bacteriotoxins in the soil, it being assumed that these toxins are decomposed by the antiseptics (chiefly toluene vapour) that we used. We have also considered the possibility of changes in the colloids and other materials that may be supposed to coat the particles of the soil.

Our experiments show that the detrimental factor has all the attributes of living organisms. Thus it is a positive factor (*i.e.* it is not a lack of some essential or desirable condition); it is capable of

growth and of extinction; once extinguished it does not arise again until some of the untreated soil is added. The difficulty of defining life is well known, but we think the sum of the properties points conclusively to a living organism. Further, every deduction we have made from the existence of the two conflicting sets of organisms has been justified by experiment, while each new experimental fact that has come to light is found to fit in readily. Our identification of the detrimental organisms with certain soil protozoa is only provisional and may be modified by subsequent zoological surveys of the soil fauna; for the present, however, we adhere to it because it accords with all the known facts.

Definite evidence could be obtained against the view that the bacterial flora is improved by partial sterilisation. The flora as a whole is certainly more effective in bringing about various decompositions, but this arises from an increase in numbers and not from an increased efficiency of the organisms. As a matter of fact the organisms lose in efficiency, and, when the old flora is put under the same conditions as the new by inoculating it into partially sterilised soil, then it attains numbers much higher than the new and brings about more decomposition (§ 24, Table X).

We have failed to find bacteriotoxins in our soils which, it should be noted, are fairly rich in calcium carbonate. Further, the deductions made from the bacteriotoxin hypothesis do not all come out right: *e.g.* the toxins ought to accumulate in partially sterilised soils where there is great bacterial activity, but they do not (§ 22). Certain of the observed facts can be explained on the hypothesis, but as new facts are brought out it becomes necessary to attribute new and more remarkable properties to the toxins in order to account for them.

There is more difficulty in dealing with the changes that might be induced in the soil colloids because it seems possible to attribute to colloids practically all the properties of living organisms. The evidence, however, seems to be against this view as a complete explanation of all the phenomena.

Of course we do not assert that bacteriotoxins do not exist in any soils or that the condition of the soil colloids plays no part in determining the bacterial population, or that partial sterilisation has no other effect on the soil except to destroy the harmful organisms. On the contrary we have shown that heating the soil to 100° C. or higher temperatures brings about considerable decomposition and considerably

alters the soil as a medium for the growth of micro-organisms<sup>1</sup>. Even the milder treatment with antiseptics does not leave the soil wholly unchanged but produces effects some of which are dealt with in a later paper. Some of our results could be explained on the supposition that heat, toluene, etc. set free some substance essential or favourable to bacteria, the lack of which in the untreated soil was limiting their numbers. But many of our results cannot be explained in this way. The only hypothesis covering all the facts is that in normal soils bacteria are not the only active organisms, but that larger organisms occur detrimental to them, and the bacterial population of the soil at any moment is determined by the mutual interaction of these conflicting groups.

## EXPERIMENTAL PART.

### I. THE EFFECT OF THE DETRIMENTAL ORGANISMS ON THE BACTERIAL NUMBERS IN THE SOIL.

§ 1. During the course of our investigations we have frequently had occasion to bring in soils from the field, submit portions to partial sterilisation processes, keep them in bottles under constant conditions of moisture and aeration at the laboratory temperature and make periodical counts of the bacteria by gelatine plate cultures. Some of the results are collected in Table I. The numbers in the untreated soils often vary in rather an erratic manner, rising and falling for no obvious reason; they show much more regularity in the partially sterilised soils, however, and generally rise steadily to a maximum at which they either remain or begin to fall; only rarely do they fluctuate as in the untreated soils.

As the partially sterilised soils had been kept alongside of the untreated soils and were equally moistened and aerated, the difference in behaviour cannot be attributed to any external cause but must be put down to some condition present in the untreated and absent from the partially sterilised soils.

<sup>1</sup> It seems impossible to convince some soil biologists that the organic matter of the soil suffers decomposition on heating to high temperatures, thereby changing the soil as a medium for the growth of organisms. Again and again we find distinguished investigators steaming soil under pressure and assuming that it has undergone no change. Conclusions drawn from experiments with these steamed soils are applied to ordinary unheated soils, not only without modification, but apparently without seeing the need for any modification. And yet for the past thirty years chemists have been giving proofs of this decomposition.

§ 2. A simple explanation is afforded by the consideration that bacteria are not the only active inhabitants of the soil but are accompanied by larger organisms detrimental to them and keeping them in check. On this view the number of bacteria in the soil at a given moment represents the balance of activity of the two sets of organisms and is therefore not connected in any simple way with the temperature, water supply, etc. These factors can increase the bacterial numbers only if they shift the balance in favour of the bacteria, and whether or not this can happen in a particular case is only discoverable on our present knowledge by actual trial.

TABLE I. *Numbers of bacteria in untreated and in partially sterilised soils.*

Millions per gram of dry soil.

	At start	End of 1st period	End of 2nd period	End of 3rd period	End of 4th period
<i>Soil 1</i> —Untreated soil .. .. .	27	16 days 10	30 days 10	74 days 45	
Soil treated with CS <sub>2</sub> * .. .	2	17	53	121	
<i>Soil 2</i> —Untreated soil .. .. .	13	15 days 9	110 days 4	170 days 9	200 days 12
Soil heated to 65° C. ....	13	21	37	45	60
<i>Soil 3</i> —Untreated soil .. .. .	11	40 days 16	100 days 9	160 days 13	500 days 6
Soil treated with toluene	2	43	41	43	18

\* In all cases it must be understood that 0.5—1 % of antiseptic is added to the soil (except where otherwise stated) and left to act for about 30 hours, and then allowed to volatilise completely. Sterilised water is then added to bring the soil to the correct degree of moistness. Throughout this paper except in Table III the bacterial numbers are stated in millions per gram of dry soil.

Small changes in conditions may therefore raise or lower the numbers to a disproportionately large extent, or, on the other hand, they may be without action. Thus in the untreated soil we expect erratic results. But in the partially sterilised soils we expect and obtain more regular results. The detrimental organisms are now killed and the surviving bacteria are free to multiply and to show the normal behaviour towards changes of temperature, etc.

*The influence of soil temperature on bacterial numbers.*

§ 3. In order to test this deduction a series of experiments was started to ascertain the effect of temperature on the numbers of bacteria

in the soil. A quantity of fresh arable soil was put through a 3 mm. sieve and divided as uniformly as possible into a number of portions which were kept in bottles plugged with sterile cotton wool. Half of the samples then received 1 per cent. of toluene which, after 24 hours, was allowed to evaporate by spreading out the soil on sterilised paper in a closed room for 30—40 hours. No smell could then be detected. The soil was returned to the bottles and received sufficient sterilised water to bring all the samples up to a uniform moisture content representing 60—70 per cent. of the saturation value. Some of the bottles were stored in a shed where the temperature was low but variable ( $5^{\circ}$ — $12^{\circ}$  C., unfortunately we had no thermostat working at  $10^{\circ}$  C.) while others were stored in incubators maintained respectively at  $20^{\circ}$ ,  $30^{\circ}$ ,  $40^{\circ}$  and  $50^{\circ}$  C. Samples were periodically taken out for analysis, the bacteriological results of which are given in Table II. Some of the results are plotted in Figs. 1 and 7.

§ 4. In the untreated rich soil (No. 2) kept at  $20^{\circ}$  the bacterial numbers rose steadily for 20 days and then fell off; at  $30^{\circ}$  the numbers fell during the whole time, while at  $50^{\circ}$  the fall was rapid and complete. In the untreated poor soil (No. 1), kept at  $20^{\circ}$ , the numbers fell off from the outset and are consistently below those in the same soil kept at a lower temperature. At  $30^{\circ}$  almost the same results were obtained except on one occasion, at  $40^{\circ}$  there is a partial drop which becomes complete at  $50^{\circ}$ . In the richest soil the same general result is obtained, the numbers fell off at  $20^{\circ}$  and are always below those in the soil kept at  $5^{\circ}$ — $12^{\circ}$ . There is some outburst of activity after the soil has been stored for three weeks at  $30^{\circ}$ , but this does not persist; at  $40^{\circ}$  there is a marked falling off. The most remarkable feature, however, is that, with one exception, the numbers are no higher at  $20^{\circ}$  or  $30^{\circ}$  than at the lower temperature, but on the contrary they are generally lower.

Thus it appears that, in these untreated soils, rise of temperature does not exert the favourable influence on bacterial numbers that might have been expected; any beneficial effect is only temporary. In other words the detrimental organisms become more active than the bacteria as the temperature rises to  $20^{\circ}$ .

A wholly different set of results, however, is obtained where the soils have been previously exposed to the vapours of toluene. In the soil *RC* the numbers steadily rise at  $20^{\circ}$  and are always much higher than at the lower temperature; the numbers also rise for a time at  $30^{\circ}$ , but do not get so high as at  $20^{\circ}$ . In the richest soil very similar

results are obtained; there is a steady rise at the low temperature and a much quicker rise at  $20^{\circ}$  (the temporary drop after 25 days we are unable to explain). At  $30^{\circ}$  the rise is even more rapid but is not maintained, while at  $40^{\circ}$  the conditions become less favourable still. The poor soil behaves like the others, except that even at  $20^{\circ}$  the high numbers cannot be maintained, but fall to 30 millions per gram. All

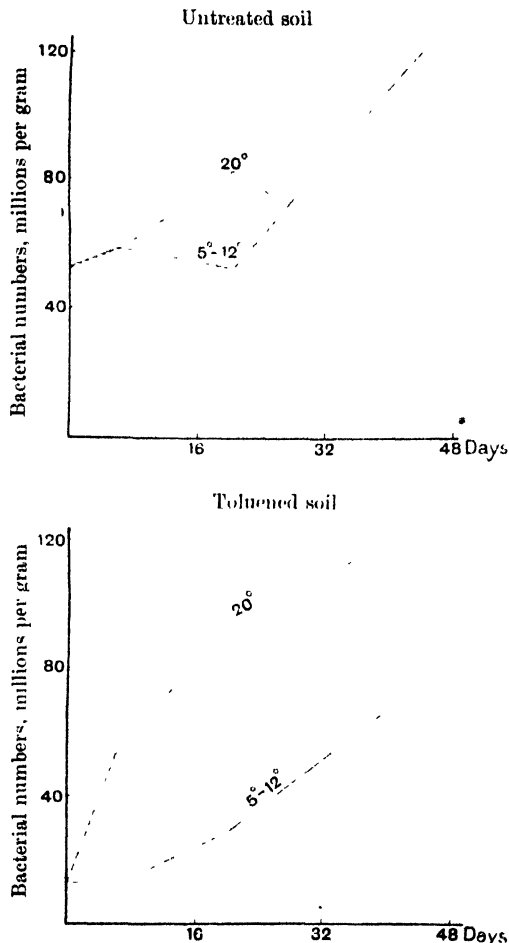


Fig. 1. Effect of varying temperatures of storage on the bacterial numbers in the soil. Soil *RC* (Table II).

these partially sterilised soils stand out in sharp contrast with the untreated soils in that there is a much more rapid increase in bacterial numbers when the temperature is raised to  $20^{\circ}$  than when it is kept

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TABLE II. *Effect of temperature on the numbers of bacteria in untreated and in sterilised soils.*

Millions of bacteria per gram of dry soil

1. A poor soil containing 14 % water, 0.18 % N, 3.16 %  $\text{CaCO}_3$  and losing 4.6 % on ignition.

Temperature, ° C.	At start	Untreated soil			At start	Soil treated with toluene		
		After 6 days	After 28 days	After 58 days		After 6 days	After 28 days	After 58 days
5°-12°	11	11	9	7	3	8	27	28
20°	11	8	4.5	6	3	50	30	30
30°	11	8	9	6	3	43	24	31
40°	11	9	2	7.5	3	12	4	6
50°	11	4	0.7	1	3	2	1	1

2. A richer soil, *RC*, containing 16 % water, 0.37 % N, 0.57 %  $\text{CaCO}_3$  and losing 11.05 % on ignition.

Temperature, ° C.	At start	Untreated soil			At start	Soil treated with toluene		
		After 6 days	After 20 days	After 44 days		After 6 days	After 20 days	After 44 days
5°-12°	53	58	52	121	13	11	30	76
20°	53	58	82	46	13	54	92	125
30°	53	49	25	24	13	60	88	87
40°	53	7	9	9	13	43	49	7
50°	53	1	13	1	13	2	10	1.5

3. A very rich soil, *OrI*, containing 40 % water, 0.63 % N, 1.9 %  $\text{CaCO}_3$  and losing 17 % on ignition.

Temperature, ° C.	At start	Untreated soil			At start	Soil treated with toluene		
		After 13 days	After 25 days	After 70 days		After 13 days	After 25 days	After 70 days
5°-12°	65	63	41	32	8.5	73	101	137
20°	65	41	22	23	8.5	187	128	182
30°	65	27	50	16	8.5	197	145	51
40°	65	14	9	33	8.5	148	52	100

The amounts of nitrogen present as ammonia and nitrate on the various dates are given in Table XVI.

The percentages of nitrogen, calcium carbonate and loss on ignition, are calculated on the air dried soil in each instance.

at 5°—12°; the bacteria being free to multiply now that the detrimental organisms are killed. It will further be noticed that the organisms counted by the method adopted cannot long survive a temperature of 50°, suffer considerably at 40°, and do not flourish as well at 30° as at 20°.

§ 5. The ineffectiveness of increased temperatures to increase bacterial numbers in ordinary untreated soils is not a peculiarity of our soils. It is seen also in the experiments of Hiltner and Störmer, of Engberding and of Conn, and has been observed also by Löhnis. The bacterial counts made by Hiltner and Störmer<sup>1</sup> at intervals during a year on field plots show no tendency for bacteria to increase as the temperature rises, the August results being no higher than those obtained in February. Some of their figures are:

Bacteria in millions per gram (Hiltner and Störmer).

	Cropped land, grass and clover	Fallow land, cultivated	
		No dung	Dung*
May 10th, 1901 .	8.3	8.0	11.0
Aug. 27th „ ...	3.2	4.2	10.5
Oct. 18th „ „	6.4	4.0	11.0
Feb. 1st, 1902 ...	6.6	4.1	9.3
June 12th „ „	8.1	5.7	7.2
Aug. 18th „ „	4.9	4.1	8.4

\* The dung was applied in July at the rate of 130 to 140 Centner pro Morgen (10 to 11 tons per acre).

Engberding<sup>2</sup> made a similar but more extensive series of counts of the bacteria in plots of ground at intervals during the year and has published his results in very complete form, giving the mean temperature of the soil during the week when each sample was taken, the moisture content, details of rainfall, etc. Here again no connection whatsoever can be traced between the bacterial numbers and the temperature, in fact it often happens on dates when the moisture contents are similar that the bacterial numbers are higher when the temperature is lower, as in the following examples:

<sup>1</sup> L. Hiltner and Störmer, Studien über die Bacterienflora des Ackerbodens, mit besonderer Berücksichtigung ihres Verhaltens nach einer Behandlung mit Schwefelkohlenstoff und nach Brache, *Arb. Biol. Abt. Land- u. Forstwirtschaft. Kais. Gesund.* 1903, Bd. 3, Heft 5.

<sup>2</sup> Diedrich Engberding, Vergleichende Untersuchungen über die Bakterienzahl im Ackerboden in ihren Abhängigkeit von ausseren Einflüssen, *Centr. Bakt. Par.* II, 1909, 23, 569—642.



	Soil temperature (mean for week) ° C.	Per cent. of moisture at time of sampling	Millions of bacteria per gram of dry soil
<i>Plot 1 a. Uncultivated and uncropped—</i>			
Aug. 14th, 1907 .....	16°·6	16·58	13·09
Sept. 26th „ .. . . .	14°·1	16·55	20·85
<i>Plot 8 b. Fallow—</i>			
April 29th, 1908 . . . .	9°·9	18·69	26·12
May 7th „ .. . . .	13°·4	17·31	14·13
June 2nd „ .. . . .	18°·9	17·87	11·77

Conn<sup>1</sup> found that the numbers of bacteria in his plots were high in February, fell in summer and rose again in autumn, and was able to draw up a very neat curve showing the changes of bacterial numbers with the season. He realises that this result implies two conflicting sets of organisms, and suggests tentatively that “there may be two groups of bacteria in the soil, one flourishing in winter, the other in summer. In this case the conflict between these two groups may explain the occurrence of two seasons, one in early fall, the other in winter, when bacteria are particularly numerous.” This hypothesis would account for Conn’s results but not ours; on the other hand, our hypothesis accounts not only for our own results but for Conn’s as well.

Löhnis<sup>2</sup> has shown that certain bacterial changes, such as the decomposition of cyanamide and urea, take place more rapidly in the soil in spring than in summer. Thus in May 1907 with a temperature of 9°, and a moisture content of 11·2 per cent., decomposition proceeded more rapidly than in the following August when the temperature was 15°·8 and the moisture content 15·8 per cent.

*The influence of moisture content in bacterial numbers.*

§ 6. Samples of soil were put up in baskets of silver wire suspended in covered beakers containing water. One sample was fairly dry, a second was moist and contained a very satisfactory amount of water for bacterial development, while the third sample just dipped into the water and was therefore wet without being waterlogged. A parallel set of three put up in the same way had previously been treated with toluene and then received sufficient water to make the percentages

<sup>1</sup> H. J. Conn, Bacteria in Frozen soil, *Centr. Bakt. Par.* II, 1910, **28**, 422—434.

<sup>2</sup> Löhnis, F. and Sabaschnikoff, A., Ueber die Zersetzung von Kalkstickstoff und Stickstoffkalk, *Centr. Bakt. Par.* II, 1908, **20**, 322—332. Other cases are quoted in Löhnis, *Handbuch der Landw. Bakteriologie*, 1910, p. 596.

equal to those in the untreated set; the water added contained an extract of the untreated soil carrying bacteria in order to make the bacterial flora as nearly as possible comparable in the two cases. The soils were all kept in the incubator at 25° C. Counts of the bacteria were made periodically by the gelatine plate method; the results are set out in Table III, and plotted in Fig. 2.

TABLE III. *Numbers of bacteria in soils containing varying amounts of water.*

Millions per gram of soil as taken from baskets, and not dried.

(a) Barnfield dunged plot.

	After 3 days	After 5 days	After 10 days	After 19 days	After 27 days
Untreated soil, dry . . .	4	4	13	6	10
„ moist . . .	14	24	37	77	20
„ saturated	26	38	38	52	40
Toluened soil, dry . . .	3	6	10	17	9
„ moist . . .	38	26	48	71	33
„ saturated	29	45	77	58	33

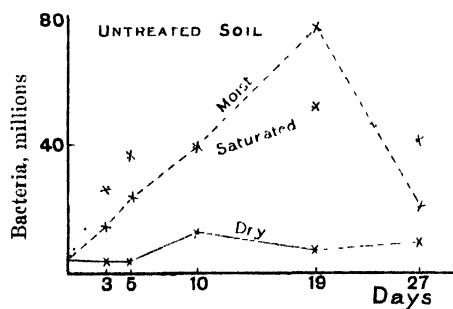
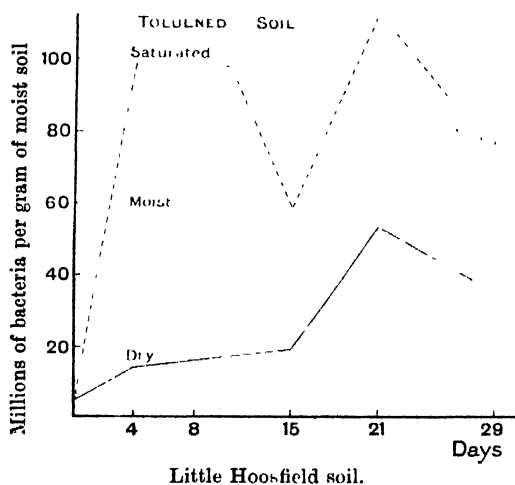
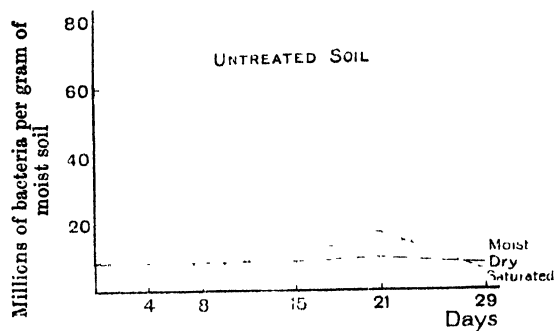
(b) Little Hoosfield.

	At start	After 4 days	After 8 days	After 15 days	After 21 days	After 29 days	Moisture present
Untreated soil, dry . . . . .	8	8	7	8	9	8	8.8
„ moist . . .	—	14	7	10	15	10	13.9
„ saturated	—	8	10	9	16	5	—
Toluened soil, dry . . .	5	15	—	20	55	37	7.4
„ moist . . .	—	58	47	—	90	80	12.0
„ saturated	—	101	98	60	116	75	—

Reference to the curves shows that in the untreated Barnfield soil (the richer of the two) there has been but little multiplication when the moisture content is low, a more rapid rate when more water is present and, for a time, a still more rapid rate when the soil is wet. But this higher rate of multiplication is only maintained for a short time. After the fifth day the increase in numbers is small and the curve bends over, showing unmistakably the operation of a limiting factor. In the tolunened soil there is no evidence of this limiting factor. The curve

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for the wet soil resembles that for the moist soil but shows a greater rate of bacterial multiplication, as one would expect. When the maximum point is reached the falling off in numbers is slower than in the untreated soil.



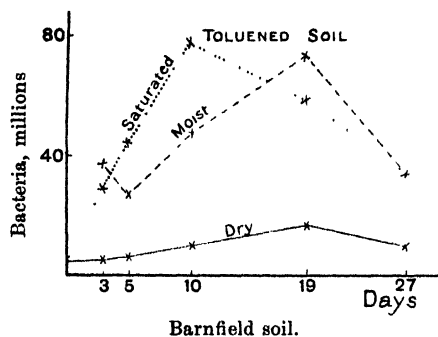


Fig. 2. Effect of variations in moisture content on the bacterial numbers in untreated and partially sterilised soils (Table III).

The addition of moisture is without effect on bacterial multiplication in the untreated Hoosfield soil where a limiting factor is plainly at work, but it leads to regular increases in bacterial numbers in the tolunened soil. Apart from two low readings on the fifteenth day, the numbers fall on to a fairly regular curve.

## II. SOME PROPERTIES OF THE DETRIMENTAL FACTOR.

§ 7. A series of experiments was now undertaken to discover some of the properties of the detrimental organisms with a view to facilitate identification. We decided, however, to work on a more general plan than was strictly necessary for this purpose, and to proceed as if we knew only the existence of a factor detrimental to bacteria but knew nothing as to its nature. We were thus enabled at once to ascertain some of its properties, to obtain still further evidence of its biological nature, and to answer some of the objections that have been raised to our previous work.

Reverting to §§ 3 and 4 and to Table II dealing with the effect of soil temperature on bacterial numbers: these results show that the factor is something positive and definite occurring in the untreated soil and not a negative factor such as lack of nutrient or other condition essential or desirable for growth. For it is difficult to see how a negative factor could cause a *drop* in the rate of multiplication at 20°; it would be more likely to set a limit so that the rate would be the same as at the lower temperature.

The moisture results of § 6, Fig. 2, Barnfield soil, also show the existence in the untreated soil of a positive hindrance to bacterial

growth. The falling off in numbers in the moist soil once the maximum is reached may be attributed to lack of food. But *the failure to reach the maximum* in the wet untreated soil can only be attributed to the presence of an active detrimental factor; for in the equally wet toluened soil the maximum is speedily attained.

The effect of the toluene is therefore not due to any foodstuff or stimulant, etc. that it may liberate, but to the extinction of something that was actually hindering bacterial development. (See also § 28.)

The experiments fall into three groups dealing respectively with:

1. The determination of the processes by which the detrimental factor can be put out of action, its extinction being indicated by the subsequent rise in bacterial numbers.

2. The biological nature of the factor.

3. Its non-bacterial nature.

#### METHODS OF EXTINCTION.

§ 8. (*a*) *Temperature*. The detrimental factor has a fairly sharp extinction point between 55° and 60° but it is also put out of action at lower temperatures if the heating is prolonged. Table IV gives the results of bacterial counts made in soils that had been heated for specified times at given temperatures and then stored under favourable conditions of moisture, aeration, etc. It will be observed that a temperature of 56° is sufficient in the first soil, 55° is barely sufficient in the second but 65° is ample, whilst in the third case a temperature of 50° maintained for one hour temporarily threw out the factor and the same temperature maintained for ten hours apparently extinguished it.

§ 9. On the other hand we failed to find any definite extinction point at low temperatures; there is, however, considerable difficulty in cooling soil owing to its low conductivity. When the cooling was made really effective by pouring liquid air on to soil contained in a Thermos flask and leaving it there to evaporate ((*a*) in Table V) the detrimental factor was put out of action and after 14 days the bacterial numbers were 60 millions per gram in place of 29 in the untreated soil. But the suppression was only temporary, and after a time the factor reasserts itself so that at the end of 42 days the numbers of bacteria were down even below the level in the untreated soil. On the other hand, when the soil was put into narrow test tubes and immersed in liquid air for half an hour the detrimental factor did not suffer and the bacterial numbers remained the same as in the untreated soil ((*b*) and

(c) in Table V). Immersion of test tubes of soil in solid carbon dioxide for about 2 hours was sufficient in one case to suppress the detrimental factor temporarily but not in another. More prolonged immersion in ice and salt was also successful in one case but the temperature of melting ice produced no change.

TABLE IV. *Effect of heat on the detrimental factor.*

Temperature of heating	Time of heating	Millions of bacteria per gram of soil					
		At start	After 7 days	After 21 days	After 68 days	After 142 days	
Unheated	—	11	10	12	11	4	Factor not extinguished Factor extinguished
40°	—	7.5	9	10	7.5	3	
56°	—	2	14	16	37	45	
		At start	After 15 days	After 120 days	After 180 days	After 210 days	
45°	—	13	9	4	9	12	Factor not extinguished ? Factor extinguished
52°	—	15	11	9	13	23	
55°	—	5	5	3	13	73	
65°	—	13	21	37	45	60	
		At start	After 13 days	After 53 days	After 105 days	After 245 days	
Unheated	—		8	9	13	12	{ Factor suppressed but apparently not extinguished Factor temporarily extinguished
50°	1 hour		26	26	15	16	
50°	12 hours	—	15	16	36	20	

(The amounts of nitrogen present as ammonia and nitrate on the various dates are given in Table XV.)

§ 10. Our conclusion is that when the soil is cooled the detrimental factor is temporarily put out of action, and the extent to which it suffers depends on the effectiveness of the cooling. A long exposure to a moderately low temperature may be more effective than a short exposure to a much lower temperature. The factor is not permanently extinguished but reappears after a time.

§ 11. (b) *Rapid drying.* Soil was exposed in a thin layer in a hot room at 35°—38° for varying intervals and then moistened and stored in

bottles in the usual way. The results of the bacterial counts are given in Table VI; they show that 10 days' drying throws the factor out of action, but only temporarily, for the bacterial numbers fell again before 120 days had elapsed, indicating that the factor had become active once more. Another lot of soil exposed to 10 days' hot bright sunshine during June, 1911, behaved in a similar manner. There is a distinct resemblance between these effects, and those observed when soil is heated to 50° for an hour or cooled to a low temperature. In all of these cases the treatment falls somewhat short of what is wanted for complete extinction of the detrimental factor, and the result is a temporary suppression of the factor, followed by a re-establishment which appears to be complete.

TABLE V. *Effect of low temperatures on the detrimental factor.*

Cooling agent	Millions of bacteria per gram of dry soil					Ammonia* and nitrate produced	
	Approximate temperature	Length of exposure	At start	After 14 days	After 42 days	After 14 days	After 42 days
Liquid air (a)	-180° C.	½-1 hour	8	60	12	—	—
" (b)	—	"	—	26	27	—	28
" (c)	—	"	8.2	26	28	—	25
Solid CO <sub>2</sub> (a)	-60° C.	2 hours	7	43	18	23	24
" (b)	—	"	—	26	13	22	26
Ice and salt (a)	-18° C.	6 hours	7	10	33	20	24
" (b)	—	"	—	37	57	22	24
Ice (a)	0° C.	8 hours	9	21	19	21	26
" (b)	"	"	—	24	23	20	23
Untreated (a)	—	—	11	31	21	20	26
" (b)	—	—	—	27	25	22	26

In all cases the ammonia was by distillation with magnesia *in vacuo* as described in this *Journal*, 1910, 3, 233; and the nitrate by the zinc copper couple method (this *Journal*, 1912, 5, 32). The results are invariably stated as parts per million of dry soil.

\* N as NH<sub>3</sub> varies from 2 to 3 parts, showing that nitrifying organisms were unaffected.

§ 12. This result is easy to explain on the view that the factor is biological: the treatment kills many of the detrimental organisms but not all, and the survivors subsequently multiply to their original density; in the meantime, however, the bacteria have a tolerably clear field for development. The result is difficult to explain on any toxin hypothesis unless one can assume that a toxin which is incompletely decomposed has the power of reproducing itself after a time; other hypotheses appear to present similar difficulties (*e.g.* see § 30, p. 184).

TABLE VI. *Effect of rapid drying on the detrimental factor.*

Method of drying	Millions of bacteria per gram of soil					
	At start	After 30 days	After 70 days	After 120 days	After 210 days	
Arable soil						
Untreated .....	11	5	(18)	11	—	Factor not extinguished *
24 hrs at 35°-38°	5	7	18	11	7	
5 days "	4	9	17	9	9	Factor "temporarily" suppressed
10 days "	—	12	25	13	10	
10 days sunshine	2	18	22	7	11	
	At start	After 26 days	After 42 days			
Richer soil, RC.						
Untreated ...	27	28	39			
10 days at 35°-38°	7	37	59			

	N as NH <sub>3</sub> , parts per million					N as NH <sub>3</sub> and nitrate*, parts per million				
	At start	After 30 days	After 70 days	After 120 days	After 210 days	At start	After 30 days	After 70 days	After 120 days	After 210 days
Arable soil										
Untreated ...	—	—	1	1	—	19	34	24	39	—
24 hrs at 35°-38°	7	1	1	1.5	2	25	31	37	44	56
5 days "	9	1	2	1.5	2	27	37	48	48	55
10 days "	7	2	2	2.5	1.5	25	39	57	61	81
10 days sunshine	3	0.5	1.5	0.5	2.5	21	31	42	44	58
	At start	After 26 days	After 42 days			At start	After 26 days	After 42 days		
Richer soil, RC.										
Untreated .....	5	2	3			91	105	105		
10 days at 35°-38°	15	3	2			96	139	141		

\* In order to save space the nitrate figures are not given separately, but are added to the NH<sub>3</sub> figures to yield these totals. The amount of nitrate in any particular case can be readily seen by deducting the corresponding ammonia figure from the total.

§ 13. (c) *Antiseptics. Organic Liquids.* The action of organic antiseptics on the soil is not entirely simple. A small amount of ammonia is immediately produced by some process we have not yet investigated. Subsequently, after the antiseptic is completely removed from the soil,



there sets in a rapid production of ammonia which remains as such because the nitrifying organisms are killed. The bacteria growing on gelatine plates are greatly reduced in numbers by the action of the antiseptics, but they increase to a very considerable extent afterwards.

§ 14. Organic liquids that do not possess marked antiseptic properties also cause an immediate liberation of ammonia. This may be (but is not invariably) followed by an increased rate of production of ammonia which is converted into nitrate showing that the nitrifying organisms have survived. There may also be an increase in bacterial numbers. Neither the increased production of ammonia nor the increased rate of multiplication of bacteria (when these phenomena set in) is nearly so marked as in the case of strong antiseptics, and it is not clear whether this is a mild instance of the usual case, or a different type of action altogether. The simplest view is that only the liquid form of these substances is capable of killing the detrimental factor, the vapours being much less potent. As the liquid comes into contact merely with a small part of the soil the partial sterilisation effect is produced only to a restricted extent.

§ 15. Table VII shows the effect of hydrocarbons on the soil, approximately one per cent. by weight being used in each case. This was left to act for two days on the soil in tightly closed bottles and was then allowed to evaporate by spreading the soil in a thin layer for some 48 hours. The soil was then moistened and put up in bottles in the usual way. The benzene ring compounds show marked antiseptic properties, killing the nitrifying organisms and the detrimental organisms, and allowing the bacteria subsequently to multiply considerably (the xylenes did not completely volatilise from the soil and appear somewhat to have checked the subsequent bacterial development). Four parts per million of ammonia are produced immediately, and there is a subsequent development of 26 parts in 26 days.

The open chain compounds, however, belong to the second type (§ 14). They cause practically the same initial production of ammonia—5 parts per million—and there is also a subsequent production of ammonia, which, however, amounts to 12 parts instead of 26 in the 26 days. None of the compounds kills the nitrifying organisms. The subsequent effect on the bacterial numbers is variable: pentane causes no increase<sup>1</sup>, hexane a marked one, and heptane a smaller one.

Cyclohexane is intermediate in action between the open chain and the benzene ring compounds.

<sup>1</sup> The only case of this action we have observed. See footnote, p. 215.

TABLE VII. *The effect of various organic liquids on bacterial activity in the soil.*

100 grams of soil received approximately 1 gram of liquid. Soil from Lucerne ley contained 12% water, 0.13% N, 1.8% CaCO<sub>3</sub>, and lost 4.9% on ignition.

Hydrocarbons	Bacteria after 26 days, millions per gram of dry soil	Effect on nitrification	Parts per million of dry soil		
			NH <sub>3</sub> immediately produced	NH <sub>3</sub> in soil after 26 days	NH <sub>3</sub> and nitrate produced in the 26 days
Untreated .....	10.4	—	—	2	4
Open chain—					
Pentane...	11.3	unaffected	5	1	12
Hexane ..	33.6	"	5	2	13
Heptane .....	17.1	"	5	1	12
Ring—					
Cyclohexane. ....	lost	much inhibited	6	15	17
Benzene ring—					
Benzene ..	57.3	entirely suppressed	4	33	26
Toluene ..	60.0	"	4	34	26
<i>o</i> -Xylene ..	37.1	"	4	29	23
<i>m</i> -Xylene ..	30.4	"	4	27	19
<i>p</i> -Xylene ..	35.3	"	4	23	12
			NH <sub>3</sub> and nitrate produced in 27 days		
			After 27 days		
Untreated .....	10.5	—		4	
Methyl alcohol....	20.7	unaffected		0	
Ethyl alcohol ..	29.5	"		6	
Tertiary butyl alcohol.	66	much inhibited		34	
Amyl alcohol ..	105	entirely suppressed		17	
Other substances—					
Untreated ..	10.4	—		4	
Acetone .....	14.3	unaffected		0	
Ether .....	30	somewhat checked		19	
Petrol .....	32.4	unaffected		10	
Alicyclic derivatives					
Untreated. ....	14.2	—		3	
Bases—					
Pyridene *	250	entirely suppressed		154	
Collidene *	4	"		430	
Lutidene *	6.6	"		196	
Other substances—					
Thiophen .....	65	entirely suppressed		32	
Toluene .....	68	"		31	
Nitrobenzene *	5	"		7	
Benzaldehyde *	37	"		0	

\* These substances could not be removed from the soil by volatilisation.

(The rest of the Table is on p. 173.)

§ 16. The alcohols also fall into two groups, methyl and ethyl alcohols being relatively ineffective, while tertiary butyl and amyl alcohols are of the same order of effectiveness as toluene. Further investigation is necessary to decide whether the action is precisely the same as that of toluene, because of the possibility that the traces of alcohol left in the soil may serve as food for the bacteria and so bring about an increase in numbers; it is difficult otherwise to account for the 105 millions of bacteria in the soil treated with amyl alcohol.

Acetone is apparently inert; ether is distinctly active but not nearly so potent as toluene; petrol is less active. In another experiment on another soil both ether and chloroform were practically as effective as toluene.

All these substances, like the hydrocarbons, cause a small immediate liberation of ammonia from the soil.

§ 17. Pyridene behaves in a remarkable manner, causing an enormous rise in bacterial numbers and in the amount of ammonia present in the soil. It was impossible to remove all the pyridene by simple evaporation and a certain amount remained in the soil; the very high amounts of ammonia and bacteria indicate that some of this has been decomposed. This result has been obtained on several occasions and always with the purest pyridene obtainable from Kahlbaum; it is the more striking in that pyridene is very stable to ordinary reagents, almost entirely resisting the attack of nitric acid, sulphuric acid, the halogens, etc. Collidene and lutidene also give rise to large amounts of ammonia in the soil. The manurial value of pyridene has been demonstrated in an earlier experiment<sup>1</sup>.

Thiophen behaves exactly like toluene. This result is obtained only when the soil is in so fine a condition that the toluene vapour can penetrate freely. In pot experiments where the soil is lumpy thiophen may prove more effective<sup>2</sup>.

Nitrobenzene and benzaldehyde could not be removed from the soil.

§ 18. *Inorganic antiseptics.* There is considerable difficulty in securing uniform distribution of inorganic antiseptics in the soil because of their non-volatile nature; we have therefore confined ourselves to those that give off poisonous gases. Bleaching powder, calcium sulphide, and hydrogen sulphide in moderate quantities all behave like toluene as shown in Table VII, while sulphur dioxide (in moderately strong dose), bromine, and flowers of sulphur, all proved too drastic under the

<sup>1</sup> E. J. Russell and F. R. Petherbridge, this *Journal*, 1912, **5**, 106.

<sup>2</sup> *Loc. cit.* p. 110.

conditions of the experiment; whether in smaller quantities they would behave like calcium sulphide we did not determine.

TABLE VII (cont.). *The effect of various inorganic antiseptics on bacterial activity in the soil.*

Arable soil containing 15 % water, 0.18 % N, 3.16 %  $\text{CaCO}_3$  and losing 4.6 % on ignition.  
100 grams of soil received approximately 0.25 gram of antiseptic.

	Bacteria after 30 days, millions per gram of dry soil	Effect on nitrification	Parts per million of soil	
			$\text{NH}_3$ immediately produced	$\text{NH}_3$ and nitrate produced in the 30 days
Untreated .....	8.6	—	—	12
Calcium sulphide...	42.5	entirely suppressed	1	18
Hydrogen sulphide	64.5	?	9	16
Bromine .....	4.3	?	8	3
Flowers of sulphur	4.3	suppressed	0	0
Untreated .....	11.3	—	—	23
Sodium sulphite ...	10.3	unaffected	1	18
Bleaching powder	32	entirely suppressed	8	34
Sulphur dioxide ...	0	"	0	5

The addition of one per cent. of quicklime was also found to partially sterilise the soil, producing the same kind of effect as toluene and other agents. It caused at first a depression in bacterial numbers, and also in the nitrifying organisms, but later on, when it was converted into carbonate, the usual increase took place both in numbers and in the amount of decomposition. This forms the subject of a later communication.

§19. All the experiments in which the antiseptic was removed from the soil lead to the same conclusion. Whenever the substance used is sufficiently potent to kill the nitrifying organisms it also puts the detrimental factor out of action, so that after it is removed from the soil, the numbers of bacteria and the rate of production of ammonia both increase to a marked extent. To this rule there is no exception.

To the converse statement there are exceptions: distinct increases in bacterial numbers and rates of ammonia production are sometimes obtained even when the nitrifying organisms are not all killed. The increases are not as marked as before, and the cases can all be explained on the view that the vapours of these substances are much less poisonous to micro-organisms than the liquid states.

We may conclude that those substances only which are capable of acting as antiseptics in the soil possess the power of suppressing the harmful factor.

§ 20. *The mode of action of the toluene.* The extinction of the harmful factor by toluene is complete even when only small quantities of toluene are used. There was no consistent difference in the bacterial population of portions of a poor soil that had been treated with 0.25, 0.5, 1, 2 and 4 per cent. respectively of toluene (Table VIII). 0.25 per cent. proved wholly insufficient to "wet" the soil, 4 per cent. on the other hand "wetted" it completely. It thus appears that the action is between the vapour and the soil, not the liquid and the soil.

In the richer soil 0.25 per cent. of toluene proves equally effective with the others for 30 days but not for 74 days. It was clear, however, that the vapour had not penetrated the whole of the soil as some of the nitrifying organisms escaped and set up a brisk nitrification after some 20 or 30 days had elapsed. Even 1 per cent. did not wholly exterminate these organisms but it so depressed them that they did not produce any measurable amount of nitrate till after the 30th day.

0.25 per cent. of carbon disulphide also proved insufficient to penetrate the soil, as seen by the fact that it only reduces the initial bacterial numbers to 9 instead of 2 millions per gram. It is therefore less effective than 1 per cent., but on the other hand, 1 per cent., which does not thoroughly "wet" the soil, is *more* effective than 4.4 per cent. which does. The conclusion to be drawn is that action is complete when the vapour has reached all parts of the soil, this point being indicated by the immediate depression of the bacterial numbers to the minimum number represented by the spores, and the suppression of the nitrifying organisms. The extinction of the harmful factor goes alongside with the extinction of the living bacteria and we never get one without the other; the completeness of the process can be accurately gauged by the initial bacterial counts (cf. 0.25 per cent. of CS<sub>2</sub> in Table VIII) and by determinations of the amounts of nitrate subsequently formed. This close relationship is precisely what would be expected if the extinction of the harmful factor is a process of killing, *i.e.* if the harmful factor is a living organism.

§ 21. If, however, we suppose that the harmful factor is some physical or chemical condition which is changed to a beneficial factor by antiseptic vapours we must note (1) that once the soil is penetrated the action is not proportional to the mass of the toluene and is therefore irreversible (this is also demonstrated in the next paragraph in

TABLE VIII. *Effect of varying quantities of antiseptics on the bacterial numbers and rate of ammonia production in soils.*

(a) Toluene. Arable soil as before (p. 173, Table VII).

Quantity of antiseptic used per 100 of soil	Millions of bacteria per gram of dry soil		
	At start	After 30 days	After 80 days
None	11	8	9
0.25	2.7	47	52
0.5	2.3	35	44
1.0	2.9	36	57
2.0	2.5	36	45
4.0	2.7	35	43

Richer soil, *RC*, containing 23% water, 0.37% N, 0.57%  $\text{CaCO}_3$ , and losing 11.05% on ignition.

		After 16 days	After 30 days	After 74 days
None	27.5	10	10	45
0.25	4.0	29	29	91
1.0	3.5	24	26	132
4.0	2.5	31	34	114

(b) Carbon disulphide. Richer soil, *RC*.

None	27.5	10	10	45
0.25	9.0	27	17	78
1.0	1.6	17	53	121
4.4	2.3	16	32	92

(a) Toluene. Arable soil containing 12% water.

Quantity of antiseptic used per 100 of soil	N as $\text{NH}_3$ , parts per million			N as $\text{NH}_3$ & nitrate, parts per million		
	At start	After 30 days	After 80 days	At start	After 30 days	After 80 days
None	2	4	2	23	25	30
0.25	10	26	27	25	42	44
0.5	7	26	27	24	44	45
1.0	9	25	29	25	40	47
2.0	11	25	27	27	42	45
4.0	9	29	37	23	42	58

Richer soil, *RC*, containing 23% water.

		After 16 days	After 30 days	After 74 days		After 16 days	After 30 days	After 74 days
None	4	5	3	3	80	105	102	114
0.25	—	35	35	20	—	116	124	157
1.0	—	40	43	38	—	114	117	130
4.0	—	39	43	67	—	123	126	155

(b) Carbon disulphide. Soil *RC* containing 23% water.

None	4	5	3	3	80	105	102	114
0.25	—	29	33	62	—	105	109	140
1.0	—	37	40	77	—	111	114	152
4.0	—	39	44	55	—	114	118	130

another way), (2) that the action is rapid and does not require the addition of energy. It follows for both reasons that the new state is more stable than the old. We shall see that other experiments lead to precisely the opposite conclusion (§ 30). This hypothesis therefore leads to contradictory results.

*The biological nature of the detrimental factor.*

This is further demonstrated by the experiments recorded in the following sections

§ 22. *Irreversibility of the extinction process.* When the factor is put out of action it does not reappear. The bacterial numbers in the toluened and heated soils maintain their high level (Tables I and IV). Further, a soil that has once been treated with toluene is not altered by re-treatment after a long interval. Table IX shows the results of experiments in which soil was partially sterilised and kept moist and aerated, but free from infection for many months so that active bacterial change might go on; at the end of the period the soil was divided into two parts, one of which was re-treated with toluene while the other was not. The soils were again stored under identical conditions of moisture, aeration, etc. and periodically subjected to analysis.

The immediate effect of the re-treatment is to bring the bacterial numbers down to a comparatively low level as happened after the original treatment. When the toluene is evaporated and the soil moistened the numbers begin to rise, but they only slowly attain their previous level and never much exceed it; the change is thus altogether different from that occurring when an untreated soil is toluened. It will be noticed also that treatment of a previously heated soil has no permanent effect on bacterial numbers, the toluene having done nothing that was not already done by the heat: certain differences have, however, been observed in other soils.

§ 23. The factor is therefore not produced in soils kept free from reinfection; once it is removed it does not reappear. It is therefore neither a bacterial product nor any consequence of bacterial action, for if it were it should accumulate to a recognisable extent in the partially sterilised soils where large numbers of bacteria have been present over long periods.

*The new flora compared with the old. The non-bacterial nature of the detrimental factor.*

§ 24. The bacterial flora of the partially sterilised soil is simpler than that of the untreated soil, but the missing groups can be introduced

TABLE IX. *Effect of re-treating partially sterilised soils after a long interval.*

Date of original treatment	Date of re-treatment	Millions of bacteria per gram			N as NH <sub>3</sub> , parts per million			N as NH <sub>3</sub> and nitrate, parts per million		
		At time of re-treatment	After 45 days	After 100 days	At time of re-treatment	After 45 days	After 100 days	At time of re-treatment	After 45 days	After 100 days
Aug. 17th, 1908	July 13th, 1910	Soil untreated .....	14.5	15	2	1	1	44	25	80
		Treated with toluene .....	70	57	5	2	2	91	107	112
		Treated with toluene, and then re-treated ..	60	52	5	21	17	91	109	112
May 29th, 1909	Oct. * 27th, 1911	Arable soil as before—								
		Untreated .....	8	11	8	—	1	—	—	84
		Untreated, and then treated with toluene ..	1.4	53	37	—	18	—	—	100
		Treated with toluene .....	39	43.5	36	—	1	—	—	89
		Treated with toluene, and then re-treated ..	15.4	48	46	—	12	—	—	100
Nov. 4th, 1911	Dec. 21st, 1911	Heated to 98° .....	36	67	38	—	0	—	—	89
		Heated to 98°, and then treated with toluene	11	61	44	—	5	—	—	92
		Soil RC (see Table VIII)—								
		Untreated .....	66	60	28	4	3	108	111	125
		Treated with toluene .....	120	118	62	24	7	122	123	—
		Treated with toluene, and then re-treated ..	—	60	30	...	46	49	136	141
		Treated with CS <sub>2</sub> .....	110	109	36	44	48	127	129	—
		Treated with CS <sub>2</sub> , and then re-treated .....	—	67	60	...	53	50	129	131

\* The percentage of nitrogen was determined in these soils on Jan. 30th, 1912, i.e. 100 days after re-treatment, and was found to be—

Treatment on May 29th, 1909      N% on Jan. 30th, 1912

Untreated .....	...	...	...	...	152
Heated .....	...	...	...	...	143
Treated with toluene which was then allowed to evaporate	...	...	...	...	139
Treated with toluene which was then left in .....	...	...	...	...	145

This relative loss of nitrogen from partially sterilised soils is quite usual (see this *Journal*, 1909, 3, 126, also 1912, 5, 38, 99).



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by infecting with a little of the untreated soil, or its water extract. Reference to Table X shows that the added bacteria develop side by side with those already present in the toluened soil, and there is nothing to indicate that any antagonism exists between the two groups. Now the added organisms include those killed by toluene; the flora in the infected soil therefore approximates qualitatively to that normally present in the untreated soils. But this infected soil flora is always more numerous than that in the toluened soil, and it follows that the flora setting up after partial sterilisation is less able to attain high numbers than the original flora, other conditions being the same. The high bacterial numbers associated with partially sterilised soils are therefore not the result of any improvement in the bacteria themselves.

§ 25. This conclusion rules out all hypotheses based on the assumption that partial sterilisation causes the bacteria to multiply more rapidly either by imparting some stimulus or by removing certain groups of bacteria which somehow prevent the others from multiplying<sup>1</sup>. It is seen on the contrary that partial sterilisation adversely affects the multiplying power of the bacteria, and the increased numbers follow, not because of the change in type, but in spite of it. The harmful factor is in short associated with something external to the bacteria.

§ 26. Two interesting facts are brought out in Table X. It is clear that the bacteria on the toluened soil do not occupy the whole ground even when they have attained their maximum numbers because there is still room for other organisms. In Exp. 1, for example, the

<sup>1</sup> It is sometimes stated that the new flora has a more favourable effect on the accumulation of plant food in the soil than the old because the denitrifying organisms are killed during partial sterilisation. This statement has several times been disproved but is nevertheless constantly reappearing. We have made numerous experiments on the subject and find that the denitrifying organisms, like the rest, increase after partial sterilisation. As examples, two experiments may be quoted in which soil was inoculated into Giltay's solution; the following amounts of nitrate were destroyed:

	Experiment 1			Experiment 2		
	After 24 hours	After 40 hours	After 64 hours	After 20 hours	After 30 hours	After 40 hours
Untreated soil .....	3.9	13.5	13.0	2.0	4.9	7.2
Soil treated with toluene ..	8.1	13.5	13.3	5.6	8.6	14.3
Soil heated to 100° C. ....	0	10.0	13.8	—	—	—
Soil heated to 56° C. for 4 hours	—	—	—	3.1	6.8	12.6

It has already been shown that the rate of loss of nitrogen from the soil (presumably as gas) is greater in partially sterilised than in untreated soils; see footnote, p. 177.

TABLE X. *Effect of introducing untreated soil into partially sterilised soils.*

Millions of bacteria per gram of dry soil.

	Experiment 1				Experiment 2				
	At start	After 40 days	After 103 days	After 160 days	At start	After 28 days	After 110 days	After 200 days	After 320 days
Arable soil—									
Untreated soil .....	11	16	9	13	—	14	9	12	8
Toluened soil .....	2.2	43	41	43.5	—	59	71	81	85
Toluened soil + 0.5 % of untreated soil ..)	—	60	71	47	—	85	54	103	64

Experiment 3		
	At start	After 45 days After 95 days
Untreated soil .....	7	11 12
Toluened soil ..	2.4	52 41
Toluened soil + water extract of untreated soil..	2.2	69 94

	Experiment 4 Richer soil, RC			Experiment 5 Very rich soil, OxL		
	At start	After 21 days	After 115 days	At start	After 15 days	After 115 days
Untreated soil ..	9	36	19	70	82	45
Toluened soil *	3.6	73	32	—	245	185
Toluened soil + 0.5 % of untreated soil ..)	—	122	70	—	287	222
Toluened soil + water extract of untreated soil ..)	—	131	53	—	409	250

\* In Experiments 4 and 5 the action of toluene was incomplete as nitrification began after the 20th day (see p. 205).

The amounts of nitrogen present as ammonia and nitrate on the various dates are given in Table XIV.

toluened soil supports some 43 million bacteria per gram and no more: but as soon as fresh groups are introduced from the untreated soil another 30 million organisms find room. Some interesting questions are thus raised to which we hope to revert on another occasion. The second fact and one we must now consider a little more fully is that the bacterial numbers fall off in the infected soils even where the numbers in the uninfected soils have remained constant. This is seen

clearly in Exps. 1 and 2 but it is masked in Exps. 4 and 5 by a fall in numbers on the tolunened soil. The experiment necessitates the introduction into our partially sterilised soils of some of the untreated soil and therefore of any of the harmful factor that might be present.

§ 27. *The introduction of the harmful factor into partially sterilised soil.* A detailed series of experiments was arranged to study the effect of introducing untreated soil containing the harmful factor into partially sterilised soils, the method adopted being to mix known proportions of untreated soil with the partially sterilised soil and make periodical bacteriological analyses. Typical results are shown in Table XI and in Fig. 3, from which it appears as before that the immediate effect of the admixture is to increase the bacterial numbers. In Exp. 1 high numbers are maintained where 0.5 per cent. but not where 5 per cent. of untreated soil is added. In Exp. 2 the numbers are all high for the first 21 days but they subsequently fall off considerably where untreated soil is present, in all but one instance becoming much less than numbers

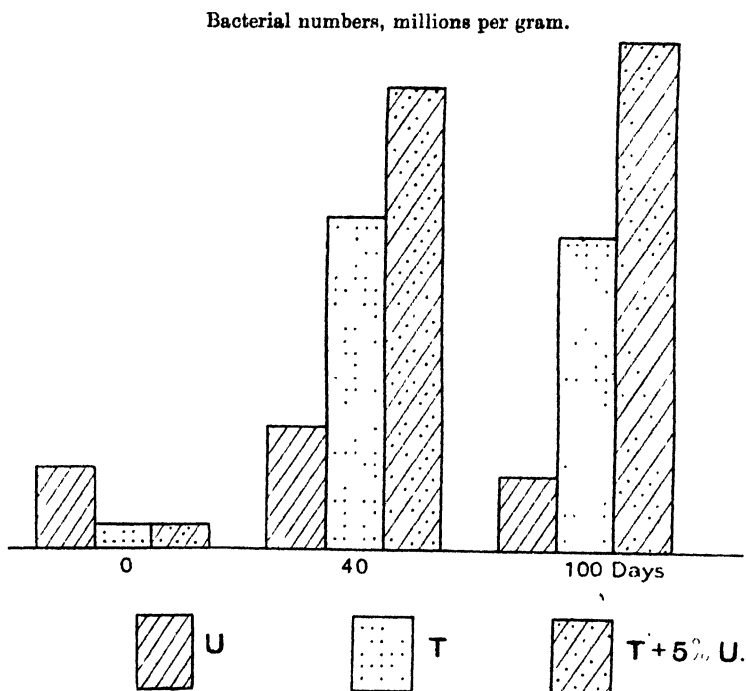


Fig. 3. Columns showing the effect on bacterial numbers of introducing untreated soil into partially sterilised soils. (a) 1st period, where an increase is produced through the activity of the added bacteria.

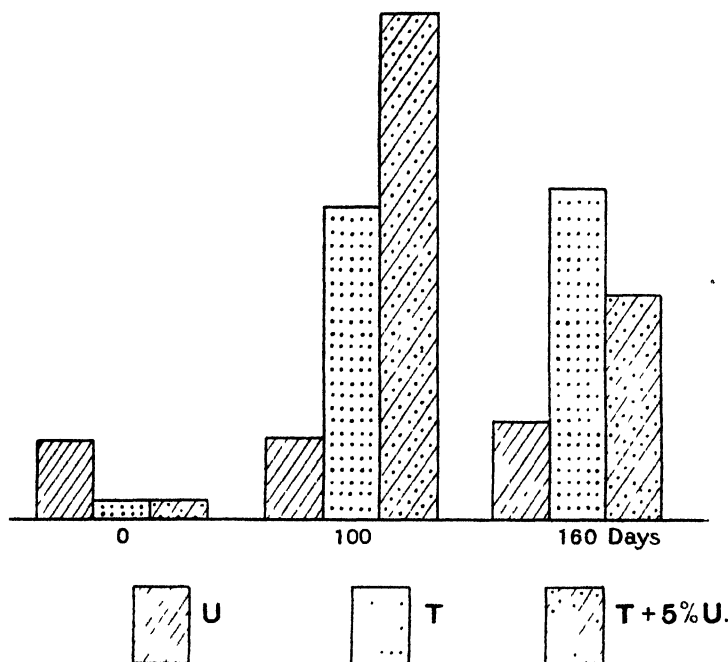


Fig. 3 (continued). (b) 2nd period, where a depression is produced through the slower development of the harmful organisms (Table XI, Exp. 3).

calculated from the proportions of tolunened and untreated soil on the assumption that the soils are inert to one another. In all experiments this divergency is noticed after a longer or shorter period; the final counts in Table XI and the calculated figures are as follows:

Per cent. of untreated soil present		0.5	5	20	50	100
Exp. 1.	Calculated numbers	74	71	—	—	
	Observed	115	61	—	—	14
Exp. 2.	Calculated numbers	44	42	36	25	
	Observed	28	16	29	35	7
Exp. 3.	Calculated numbers	43	41	37	28	
	Observed	47	35	23	16	13
Exp. 4	Calculated numbers *	—	220	191	—	
	Observed	231	173	148	—	37
Exp. 5.	Calculated numbers	201	192	166	111	
	Observed	74	170	122	28	20

\* From tolunened soil infected with 0.5 % untreated soil (see note to Exp 4 on Table XI).

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**TABLE XI.** *Effect on bacterial numbers of introducing untreated soil into partially sterilised soils and vice versa.*

Millions of bacteria per gram of dry soil.

**Experiment 1.** Arable soil as before.

(a) Untreated soil added to partially sterilised soil.

	At start	After 50 days	After 100 days
Toluened soil.....	2.1	71	74
" " + 0.5% untreated soil ...	3.2	103	115
" " + 5% " " ..	4	58	61

(b) Partially sterilised soil added to untreated soil.

Untreated soil .....	8	17	14
" " + 0.5% tolueued soil ..	8	14	17
" " + 5% " " ...	5	11	14

(a) Untreated soil added to partially sterilised soil.

	Exp. 2. Arable soil				Exp. 3. Arable soil			
	At start	After 7 days	After 21 days	After 58 days	At start	After 39 days	After 100 days	After 157 days
Toluened soil .....	2.9	41	66	44	2	43	41	43
" " + 0.5% untreated soil	—	76	72	28	—	60	71	47
" " + 5% " " ..	—	118	120	16	—	60	66	35
" " + 20 parts* " " ..	—	67	102	29	—	45	15	23
" " + 50 parts* " " ..	—	64	61	35	—	18	25	16

(b) Partially sterilised soil added to untreated soil.

Untreated soil.....	10	9	16	7	11	16	9	13
" " + 5% tolueued soil ..	—	19	16	8	—	23	17	15
" " + 20 parts* " " ..	—	22	36	10	—	16	17	17

	Exp. 4. Richer soil			Exp. 5. Very rich soil		
	After 33 days	After 83 days	After 170 days	After 15 days	After 69 days	After 154 days
Toluened soil.....	103	90	†	152	225	202
" " + 0.5% untreated soil	97	80	231	233	255	74
" " + 5% " " ..	104	51	173	243	117	170
" " + 20 parts* " " ..	123	84	148	267	171	122
" " + 50 parts* " " ..	—	—	—	136	74	28
Untreated soil .....	33	33	37	101	83	20

The initial counts in Exps. 4 and 5 and that marked † were lost through liquefaction of the plates.

The composition of the soils and the amounts of nitrogen present as ammonia and nitrate on the various dates are given in Table XIV.

\* Parts per 100 of mixture, i.e. 80 of tolueued + 20 of untreated soil or *vice versa* and 50 of tolueued + 50 of untreated soil; the figures are used in this way throughout the Table.

§ 28. Now we know that these calculated numbers are too low because they take no account of the increase in bacterial numbers that follows introduction of untreated soil into toluened soil, but even so the observed numbers come out still lower. Thus the effect of introducing 5 per cent. or more untreated soil into partially sterilised soil is first to increase and then after a time to considerably reduce the bacterial numbers, in some cases bringing them down near to the level of the untreated soil.

It might be argued that the high bacterial numbers first induced by additions of untreated to partially sterilised soils exhaust the supply of some essential nutrient set free by the toluene and thus inevitably lead to a reduction in numbers. In this way some of the results of Table XI might be explained (*e.g.* Exp. 2, and parts of Exps. 3 and 4) without assuming that any detrimental organisms come into play. On the other hand, Exps. 1, 5, and parts of 3 and 4 cannot be thus explained, and the only hypothesis that covers all the results is that the harmful factor has been transmitted to the partially sterilised soils. We thus have further proof that the factor is something positive and is not a negative state such as lack of a stimulant or essential requirement. The second part of the experiment also affords evidence that the untreated soil is not inert but contains a positive detrimental factor: the addition of as much as 20 per cent. of partially sterilised soil to untreated soil fails to increase the bacterial numbers, excepting temporarily and to a small extent. (See also § 7.) Other examples of depression of bacterial numbers in partially sterilised soils by infecting with untreated soils are given in Table XVII (p. 217). The partially sterilised soil B in that Table was mixed with an equal weight of untreated soil and left for 5½ months. At the end of that time the soils were mixed with hay dust: the bacterial numbers subsequently found were in millions per gram:

	Just before addition of hay	7 days after addition of hay	74 days after addition of hay
Toluened soil .....	31	175	136
Toluened soil + equal weight of untreated soil .....	25	86	66
Untreated soil .....	7	94	62

The mixed soil is now indistinguishable from the untreated soil, and the advantage of partial sterilisation has wholly disappeared.

§ 29. The harmful factor is not invariably transmitted to the same extent from the untreated to the partially sterilised soil and in a few cases indeed it is not transmitted at all. The falling off of bacterial numbers from the calculated values follows no sort of rule, being related neither to the numbers of bacteria nor to the amount of added soil. Indeed we get the same erratic changes as in the untreated soils in Tables I and II. Only rarely is the transmission so complete as to bring the numbers down to the level of the untreated soil.

§ 30. It is possible to explain these results on the supposition that partial sterilisation has effected some change in the soil colloids, making them more favourable for bacterial activity. Changes in surface tension and other properties are almost certain to take place and to react on bacterial activity. But we get into difficulties directly we suppose that this is the sole cause at work. For example: when some of the untreated soil is added the new form reverts to the old and less suitable form; addition of the new form to the old (*i.e.* of 5 per cent. of tolued soil to untreated soil) is, on the contrary, without effect. The new form is therefore less stable than the old at ordinary temperatures. This result appears to be in entire contradiction with one obtained earlier (§ 21). The supposition is also difficult to reconcile with the evidence of the active nature of the factor (§ 7) and we must therefore discard it as a satisfactory explanation of all the phenomena.

§ 31. The bacteriotoxin hypothesis does not account for the results. The depression produced by the introduction of the untreated soil ought to come into operation at once if it were caused by a toxin, and the amount of the depression should be proportional to the amount of added soil. Neither of these results is obtained. Further, as shown in our earlier paper and in Table X, the water extract of an untreated soil has no toxic effect when added to a tolued soil and not infrequently causes an increase in bacterial numbers because it itself carries bacteria. Toxic properties have been attributed to this extract by Greig-Smith<sup>1</sup> and by Bottomley<sup>2</sup>, but we are unable to obtain their results with our soils. Greig-Smith worked with soils in New South Wales which are unfortunately inaccessible to us in a fresh condition, so that we are unable to account for the discrepancy<sup>3</sup>. Bottomley used soils from the Chelsea Physic garden, but in these also we failed to find evidence of a toxin by the methods he adopted.

<sup>1</sup> *Transactions of the Linnean Society of New South Wales*, Nov. 30th 1910.

<sup>2</sup> *Report of the British Association*, 1911.

<sup>3</sup> It might arise from a difference in the amount of calcium carbonate present.

### III. THE PROPERTIES OF THE INJURIOUS FACTOR AND ITS PROBABLE NATURE.

§ 32. The properties of the injurious factor ascertained by the preceding experiments are as follows:

(a) It is permanently put out of action by toluene and other antiseptics sufficiently potent to kill nitrifying organisms, and also by heating to 55°. If the soils are kept free from reinfection it does not reappear even though the conditions are made very favourable for bacterial growth.

(b) It is temporarily put out of action by lesser degrees of heat, e.g. 50° or less, by drying for a sufficient length of time at 35°—40° and by low temperatures. After a time it manifests itself again if the soil is kept under normal conditions of temperature, water supply and aeration.

(c) It can be reintroduced into a soil from which it has been permanently extinguished by the addition of a little untreated soil.

(d) It develops more slowly than bacteria and for some time may show little or no effect<sup>1</sup>; then it causes a marked reduction in the

<sup>1</sup> This slow growth of the destructive organisms, which was emphasised in our earlier paper, vitiates some of the criticisms that have been passed on our conclusions. For example, Lipman, Blair, Owen and McLean (Experiments relating to the possible influence of protozoa on ammonification in the soil, *New Jersey Expt. Station Bull.* 248, 1912) added pasteurised and untreated soil infusions respectively to mixtures of sterilised soil (heated under a pressure of 1·5 atmospheres of steam) and dried blood. After seven days the pasteurised infusion had induced the formation of no more ammonia than the untreated infusion. They conclude that these results "do not bear out Russell and Hutchinson's contention as to the part played by protozoa in depressing the activities of soil bacteria."

It does not appear to us that the experiment really bears on the subject. In no case have we observed development of the destructive organisms in anything like so short a time as seven days. Two assumptions are also involved which the facts do not warrant: (1) the amount of ammonia formed is taken as a measure of the number of bacteria (see pp. 191 *et seq.* on this point), (2) subjecting the soil to the high temperature of steam at 1½ atmos. is supposed to leave it unchanged. The argument as we understand it reduces itself to this: the destructive organisms made no growth in seven days in a medium A (strongly heated soil), therefore they could make no growth in a longer period in a wholly different medium B (ordinary unheated soil).

In common with other soil investigators Fred (Über die Beschleunigung der Lebens-tätigkeit höherer und niederer Pflanzen durch kleine Giftmengen, *Centr. Bakt. Par.* 1912, II, 81, 185—245) assumes that heating the soil has no effect except to kill micro-organisms. He heated soil to 100° C., added ammonium sulphate and then ether, and continues "nach Russell und Hutchinson's ansicht würde dieses Antiseptikum in amöbenfreiem Boden dann keine günstige Wirkung haben" (we expressly stated in our earlier paper that we made no such claim; see also p. 156 here). No favourable action was observed, as a



numbers of bacteria and its final effect is out of all proportion to the amount originally introduced. The development is erratic and we have not learnt precisely the conditions under which it best takes place.

(e) It is shown not to be bacterial in nature (§§ 23, 25), nor a toxin (§§ 12, 23, 31), nor any adverse physical or chemical state of any of the soil constituents (§§ 21, 30) nor any negative condition such as lack of some essential or desirable factor (§§ 7, 28).

(f) It is favoured by conditions favourable to trophic life in the soil<sup>1</sup>.

(g) We see no escape from the conclusion that it is a living organism.

§ 33. In our previous paper we identified the injurious organisms with the soil protozoa and our subsequent work supports this view. Examination of a large number of soils shows that protozoa are normal inhabitants of the soil. The total number of species present must be considerable. Goodey<sup>2</sup> has examined hay infusion cultures and described a number of forms that he picked out, and Martin<sup>3</sup> has used a plate culture method and got out others. Protozoa commonly and perhaps invariably get into diseased roots wherever many bacteria have entered, but so far as we know no one has investigated them; there can be no doubt that they would amply repay study by some competent zoologist. Protozoa have been found in German soils by Hiltner<sup>4</sup>, Stürmer<sup>5</sup>, Max Wolff<sup>6</sup>, and R. H. Francé<sup>7</sup>; in the soils of Hawaii by S. S. Peck<sup>8</sup>, and of Porto Rico by Oscar Loew<sup>9</sup>. The evidence seems matter of fact, excepting only when untreated soils were treated with ether and the author admits that "Diese Beobachtung spricht für Russell und Hutchinson, doch"—he naively continues—"doch ist es möglich, und sogar wahrscheinlich, dass die gesteigerte Nitrifikation durch Äther auf einer Reizwirkung auf die nitrifizierenden Bakterien selbst beruht."

<sup>1</sup> This aspect is discussed in this *Journal*, 1912, 5, 27, 86.

<sup>2</sup> A contribution to our knowledge of the protozoa of the soil. *Proc. Roy. Soc.* 1911, 84 B, 165—180.

<sup>3</sup> A note on the protozoa from sick soils; *ibid.* 1912, 85 B, 393—400.

<sup>4</sup> Ueber neue Ergebnisse und Probleme auf dem Gebiete der landwirtschaftlichen Bakteriologie, *Jahresber. Verein für Angew. Botanik*, 1907, 5, 200.

<sup>5</sup> Die Wirkung des Schwefelkohlenstoffs auf dem Boden, *ibid.* p. 123.

<sup>6</sup> Der Einfluss der Bewässerung auf die Fauna der Ackerkrume mit besonderer Berücksichtigung der Bodenprotozoen, *Mitt. Kaiser Wilhelm Instit. für Landw.*, Bromberg, 1909, 1, 382—401; Ueber Bodenprotozoen, *Centr. Bakt. Par.* 1912, II, 33, 314—320.

<sup>7</sup> Studien über edaphische Organismen, *Centr. Bakt. Par.* 1912, II, 32, 1—7.

<sup>8</sup> Some Bio-chemical investigations of Hawaiian Soils, *Bull.* 34, *Expt. Station of the Hawaiian Sugar Planters' Association*, 1910.

<sup>9</sup> *Annual Report of the Porto Rico Agricultural Expt. Station*, 1910. Also R. Emmerich, W. Graf zu Leiningen u. O. Loew, Über Bodensauberung, *Centr. Bakt. Par.* 1912, II, 31, 466—477. Other references are given in Goodey's paper (*loc. cit.*).

to be conclusive that any of these organisms occurring in an active state would be inimical to bacteria and would therefore function as the injurious factor.

Rahn<sup>1</sup> has not only found protozoa in Michigan soils but has formed a minimum estimate of the numbers present per gram, arriving at results of the same order as our own. He further demonstrated that the numbers are considerably reduced on drying.

The great difficulty, and one that none of the seven investigators just mentioned has attempted to deal with, is to determine which forms are active and which remain as cysts under the conditions of the soil. Goodey has devoted much attention to the ciliates developing in hay infusion (Colpoda, etc.) but could find no evidence that they are active in the soil. Martin, on the other hand, considers that some of his organisms—amoebae and amoeboid forms—probably are active. Some of our observations are difficult to explain except on the view that certain protozoa are capable of growth; thus it has happened when small quantities of untreated soil have been added to toluened soil that during the first few days we failed to find either ciliates or amoebae, while later on we found them without difficulty. It is difficult to see why protozoan cysts should remain undeveloped in the soil; bacterial spores certainly show no tendency to accumulate and rarely form more than 20 or 30 per cent. of the total numbers growing on gelatine (Tables VIII, X and XI). The problem, however, is not likely to be solved until the zoological survey of the soil has proceeded further, and accurate methods devised for counting the protozoa.

§ 34. The examination of soil for protozoa is now part of our routine procedure in all experiments on partial sterilisation. Soil is inoculated into a one per cent. hay infusion and left in an incubator at 25° for 4—5 days, examination being made periodically for protozoa. No identification is attempted, but the organisms are grouped roughly as ciliates, amoebae, and monads; no attempt is made even approximately to estimate their numbers. Partial sterilisation invariably simplifies the fauna considerably, killing the ciliates and amoebae but often leaving certain monads. *Whenever the ciliates and amoebae are killed we invariably find that the detrimental factor is extinguished; whenever the detrimental factor is not extinguished the protozoa also are not killed; we have found no exception to these rules.*

<sup>1</sup> Methode zur Schätzung der Anzahl von Protozoën im Boden. *Centr. Bakt. Par.* 1913, II, 36, 419—421.

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The following are typical results obtained for heated soils:

	Bacteria after 68 days. Millions per gram of dry soil	NH <sub>3</sub> and nitrate formed after 68 days. Parts per million of soil	Detrimental factor	Protozoa found
Untreated soil	11.1	13	present	{ Ciliates Amoebae Monads
Heated to 40° for 3 hours	7.5	14.4	present	{ Ciliates Amoebae Monads
Heated to 56° for 3 hours	37.5	36.7	killed	All killed

Treatment with toluene leads to similar results:

	Bacteria after 30 days. Millions per gram	NH <sub>3</sub> and nitrate formed after 30 days	Detrimental factor	Protozoa found
Untreated soil	8	24.5	present	{ Ciliates Amoebae Monads
Toluened soil	47.4	41.6	killed	{ All killed but certain Monads

Quicklime also produces the same effect. So long as small quantities only are added the protozoa are not appreciably affected, the bacterial numbers do not fall in the beginning nor show any subsequent rise. But when a certain larger quantity has been added the partial sterilisation effect is produced, the protozoan fauna is considerably simplified and the bacterial numbers are at first depressed but later on they rise considerably and effect a corresponding production of ammonia (see § 18).

In Table XII (p. 194) it is shown that a soil stored moist in a closed bottle for 37 years still retained a complex protozoan fauna and a low bacterial population. But after treatment with toluene the protozoa were destroyed (excepting certain Monads) and the bacterial population rose considerably.

But when the soil was heated insufficiently to kill the harmful factor more of the protozoa survive:

	Detrimental factor	After 13 days		After 53 days		After 245 days	
		Bacteria millions	Protozoa	Bacteria millions	Protozoa	Bacteria millions	Protozoa
Untreated ...	Present	8	C. A. M.*	9	C. A. M.	12	C. A. M.
Heated to 50° for 1 hour...	Present but suppressed	26	C. A. M.	26	M.	16	C. A. M.
Heated to 50° for 24 hours	Temporarily extinguished	15	none	16	?	20	A. M.

\* The contractions C. A. M. stand respectively for Ciliates, Amoebae and Monads.

	At start		After 16 days		After 30 days		After 74 days	
	Bacteria millions	Protozoa	Bacteria millions	Protozoa	Bacteria millions	Protozoa	Bacteria millions	Protozoa
Untreated	27.5	C. A. M.	10	C. A. M.	10	C. A. M.	45	C. A. M.
0.3 % CS <sub>2</sub>	9	A. M.	27	C. M.	17	C. M.	78	C. A. M.
1.0 % CS <sub>2</sub>	1.6	M.?	17	M.	53	M.	121	A. M.

With a still richer soil even more marked results are obtained. In this case carbon disulphide so effectively reduced the protozoa that we could not be sure that any were left, while toluene was much less destructive. The subsequent rise in bacterial numbers was considerably higher after treatment with carbon disulphide than with toluene. The fall in numbers on adding 5 per cent. of the untreated soil is also seen to be accompanied by an appearance of a complex protozoan fauna.

	After 83 days	
	Bacteria millions	Protozoa
Untreated .....	90	C. A. M.
Treated with CS <sub>2</sub> .....	326	None found
"    "    " then mixed with 5 % untreated soil	155	C. A. M.
Treated with toluene .....	106	C. A. M.

§ 35. In several of the above instances there is evidence of an increasing complexity in the fauna as time goes on, and the simplest explanation is that there has been an actual multiplication of some of the forms which at first were present in numbers too small for us to detect. But we cannot lay too much stress on this point, as the organisms multiply rapidly in hay infusions and we are unable in our final examinations to say whether the soil we started with contained a large or a small number of organisms. This indeed is the weakness of the method. But this very weakness only makes the close connection between the destruction of the protozoa and the destruction of the harmful factor all the more striking. The survival of protozoa in other experiments unfortunately loses much of its significance because it may only mean that a small number of cysts escaped, but even here it will be noticed that the highest bacterial numbers are never attained when the fauna is complex, *i.e.* when a relatively large number of protozoa survive. In rich soils toluene often fails to kill all protozoa just as it fails to kill nitrifying organisms and to cure "sickness"; this has been traced to its low solubility and consequent inability to penetrate any but small particles of soil in presence of much moisture or organic matter<sup>1</sup>.

On the other hand, we have failed in our attempts to reduce bacterial numbers in a partially sterilised soil by introducing mass cultures of the ordinary hay infusion protozoa. Our difficulty has been to remove from the cultures the large contaminations of bacteria and bacterial food which cause disturbances directly they get into the soil; we never have a really clean experiment.

§ 36. Until a more complete zoological survey of the soil has been made it is not possible to identify the harmful organisms with certainty. We do not even know how they act; whether they devour the bacteria or whether they are present as films round the minute particles of organic matter that would otherwise serve as bacterial food, thus starving the bacterial population down to low limits. The present position in fact, is precisely that in which nitrification stood for many years; the process was known to be biological as far back as 1879, but the most diligent search among the colonies on the gelatine plate cultures then in vogue failed to bring out the organism. Not till 1891, when a new method was devised, could the organism be isolated with certainty. Our present methods of dealing with soil protozoa are those devised for dealing with pond and stream protozoa, and do not precisely

<sup>1</sup> See Russell and Petherbridge, this *Journal*, 1912, 5, 107.

reproduce the conditions obtaining in the soil. Therefore we must not at this stage lay too much stress on any relationship that comes out, but may only be accidental, between our detrimental organisms and any of the ciliates, amoebae and monads that these methods reveal. But it seems safe to draw two conclusions: (1) the detrimental organisms possess the properties of protozoa and not of bacteria; (2) the presence or absence of the detrimental organisms is intimately associated with the presence or absence of a complex protozoan fauna. We shall therefore continue to identify the detrimental organisms with the soil protozoa without, however, committing ourselves to any particular organism or set of organisms or to any rigid and exclusive definition of the term protozoa.

#### IV. THE RELATIONSHIP BETWEEN THE RATE OF PRODUCTION OF PLANT FOOD IN THE SOIL AND THE INCREASED NUMBERS OF BACTERIA BROUGHT ABOUT BY PARTIAL STERILISATION.

§ 37. *Partial sterilisation by volatile antiseptics.* It was shown in our earlier paper that the increases in bacterial numbers brought about by partial sterilisation with volatile antiseptics lead to corresponding increases in the amount of ammonia produced in the soil. Subsequent experiments have shown that this relationship does not hold universally, but ceases to manifest itself as soon as a certain amount of ammonia and nitrate has accumulated. There is a fairly well-marked limit beyond which the accumulation of ammonia and nitrate will not go, although bacterial multiplication may still continue. This limiting amount varies for different soils and is higher for soils rich in organic matter and therefore capable of retaining a considerable amount of water than for poor soils of lower water-holding capacity; it is also higher in heated than in unheated soils.

§ 38. Determinations of ammonia and nitrates<sup>1</sup> have been made in all our soils on each occasion when the bacterial numbers were estimated, and the whole of the large mass of data thus obtained can be grouped into two cases:

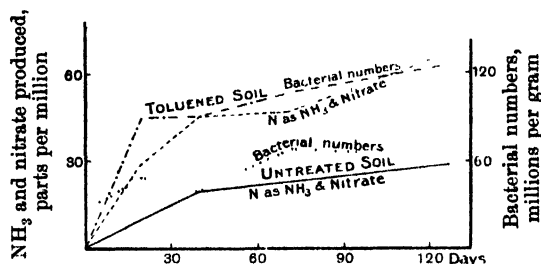
(1) Soils where the ammonia and nitrates fall well below the limit; here the increase in bacterial numbers following on partial sterilisation causes a corresponding increase in the amount of ammonia and nitrate.

(2) Soils containing much ammonia and nitrate; here the increased numbers of bacteria cause no corresponding increase in the amounts of ammonia and nitrate.

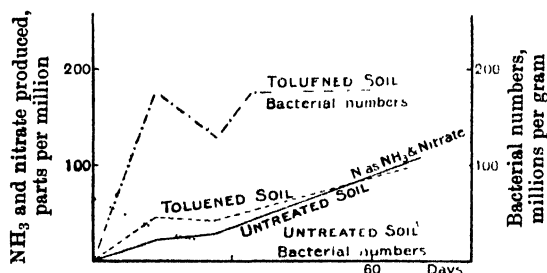
<sup>1</sup> See footnotes to Tables V and VI (pp. 168 and 169).

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These cases are illustrated by the curves in Figs. 4 and 5 showing the amounts of ammonia and nitrate, and also the numbers of bacteria present after certain intervals of time. In the diagrams illustrating Case 1 there is a sufficiently close agreement between the curves for bacterial numbers and those for the amounts of ammonia and nitrate to show the intimate relationship between these quantities. Here the highest amount of ammonia present is 45 parts per million in the



Case 1. Small amounts of ammonia and nitrates initially present (soil *RC*, Table XII (a)). A relationship is indicated between the bacterial numbers and the rate of accumulation of N as NH<sub>3</sub> and nitrate.



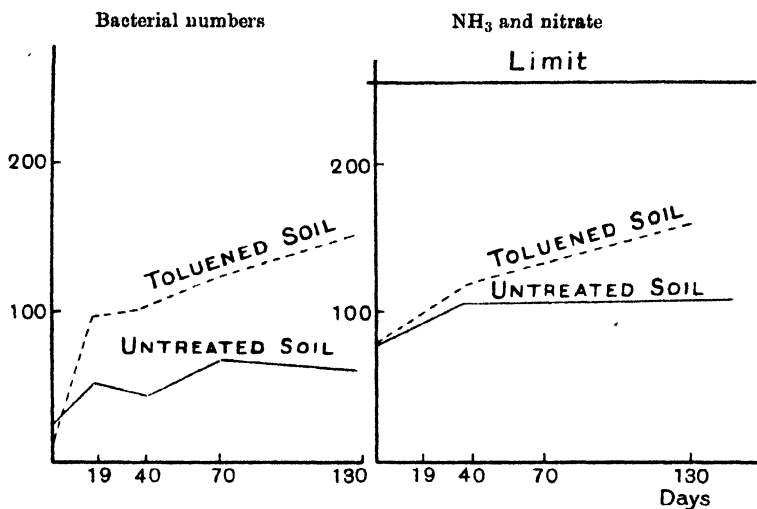
Case 2. Large amounts of ammonia and nitrate initially present (soil *OxI*, Table XII). No such relationship can be seen as in Case 1.

Fig. 4. Ammonia and nitrate produced after certain intervals of time, and also the numbers of bacteria present per gram of soil.

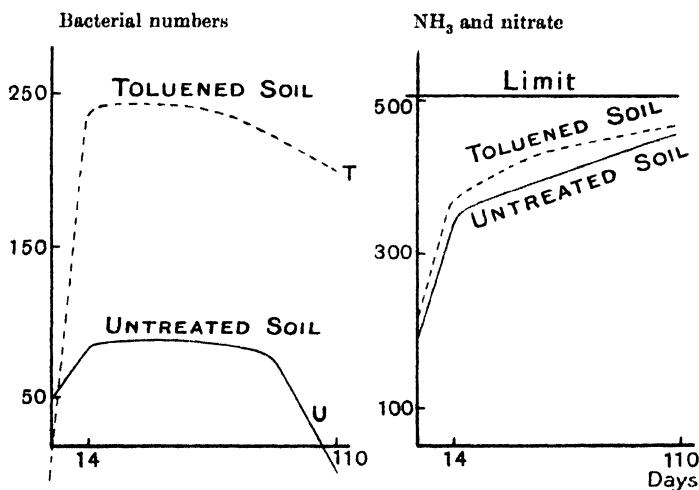
tolued soil, while the ammonia and nitrate finally amount to 150 parts per million. On the other hand, in the diagrams illustrating Case 2 there is no similarity whatsoever between the curves for bacterial numbers and the corresponding curves for the amount of ammonia and nitrate present. The production of ammonia and nitrate, in fact, proceeds at the same rate in both soils, the initial advantage gained by the tolued soil never being improved upon in spite of the large difference in bacterial numbers. But in this soil the amount of

ammonia is 180 parts per million, while the ammonia + nitrate is over 500 parts per million.

Further instances are given in Table XII.



Case 1.



Case 2.

Fig. 5. Relation between bacterial numbers and amount of nitrate and NH<sub>3</sub> formed.

§ 39. The falling off in the rate of accumulation of ammonia and nitrate is due to the ammonia and to a less extent to the nitrate already there, and not to the exhaustion of the complex precursors of ammonia



TABLE XII. *Bacterial numbers, and amounts of ammonia and nitrate in soils partially sterilised by volatile antiseptics.*

Case 1. Ammonia and nitrate in relatively low proportions at first, so that a relation can be seen with the bacterial numbers.

(a) Soil RC containing 23% moisture, 0.37% N, 0.5% CaCO<sub>3</sub> and losing 11.0% on ignition.

	Bacterial numbers, millions per gram of soil				N as NH <sub>3</sub> , parts per million of soil				N as NH <sub>3</sub> + nitrate, parts per million of soil			
	At start	After 19 days	After 40 days	After 72 days	At start	After 19 days	After 40 days	After 72 days	At start	After 19 days	After 40 days	After 72 days
Untreated .....	25	49	41	69	4	2	3	3	87	97	107	110
Treated with toluene	3	89	91	108	6	33	45	38	2	108	124	128
												149

(b) Same soil, 23% moisture.

	Bacterial numbers, millions per gram				N as NH <sub>3</sub> , parts per million of soil				N as NH <sub>3</sub> + nitrate, parts per million of soil			
	At start	After 16 days	After 30 days	After 74 days	At start	After 16 days	After 30 days	After 74 days	At start	After 16 days	After 30 days	After 74 days
Untreated .....	27	10	10	45	4	5	3	3	80	105	102	114
Treated with toluene	4	29	29	91	7	35	35	20	—	116	124	157
" " CS <sub>2</sub> .....	9	27	17	78	6	29	33	62	—	105	109	140

(c) Arable soil containing 14% moisture, 0.18% N, 3.16% CaCO<sub>3</sub>, and losing 4.6% on ignition.

	Bacterial numbers, millions per gram				N as NH <sub>3</sub> , parts per million of soil				N as NH <sub>3</sub> + nitrate, parts per million of soil			
	After 5 days	After 27 days	After 57 days	After 74 days	After 5 days	After 27 days	After 57 days	After 74 days	After 5 days	After 27 days	After 57 days	After 74 days
Untreated stored at 5°-19°	11	9	7	7	1	2	1	1	26	27	27	27
" " 20°	8	5	6	6	2	1	1	1	24	32	32	31
Toluened " 5°-19°	8	27	28	28	13	19	23	23	32	43	43	45
" " 20°	50	20	30	30	19	18	7	7	39	44	44	45

TABLE XII—Continued.

Case 2. Ammonia and nitrate in high proportions so that no relation can be seen with bacterial numbers.

(a) Soil *OxL* containing 40 % moisture, 0.67 % N, 1.9 %  $\text{CaCO}_3$  and losing 17 % on ignition.

	Bacterial numbers, millions per gram of soil				N as $\text{NH}_3$ , parts per million of soil				N as $\text{NH}_3$ + nitrate, parts per million of soil			
	At start	After 13 days	After 25 days	After 70 days	At start	After 13 days	After 25 days	After 70 days	At start	After 13 days	After 25 days	After 70 days
Untreated stored at 5°–12°	65	63	41	32	14	25	22	18	4	372	391	390
" " 20° ...	65	41	22	23	14	23	22	16	2	372	398	397
Toluened " 5°–12°	8	73	101	137	87	94	101	197	116	428	439	438
" " 20° ...	8	187	128	182	81	130	123	180	99	428	473	462

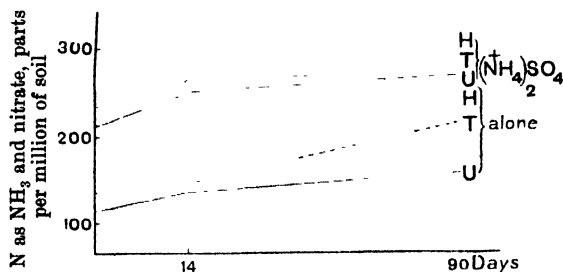
A relationship is indicated at 5°–12° but not at 20°.

(b) Soil from Agdell field bottled moist in 1874, left unopened till November 1911, *i.e.* during 37 years, then divided into three portions. Moisture on opening bottle=11.6 %.

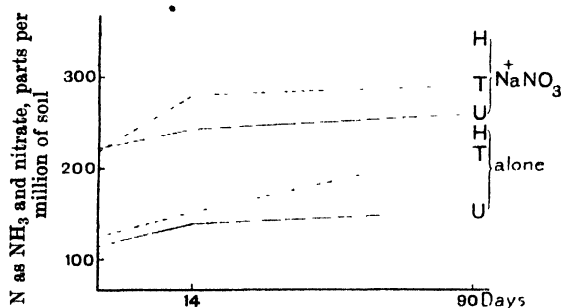
	Bacterial numbers, millions per gram				Protozoa after 33 days		N as $\text{NH}_3$		N as $\text{NH}_3$ + nitrate	
	At start	After 33 days	After 46 days	After 125 days			At start	After 125 days	At start	After 125 days
Untreated	3	4	5	8	C.M. M. M.		0	0	203	207
Treated with toluene	3	15	20	38			0	7	200	200
" " $\text{CS}_2$	2	61	46	62			0	6	200	208

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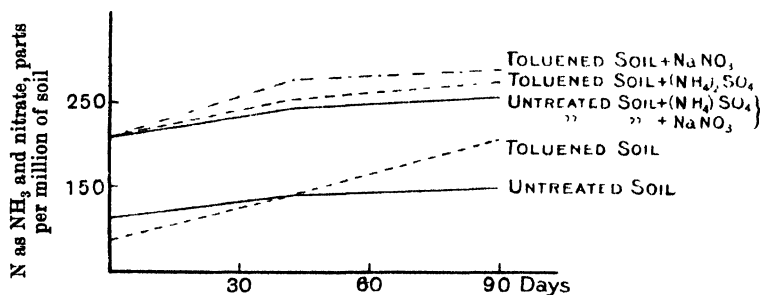
in the soil, since it can be brought about simply by adding suitable quantities of ammonium sulphate and sodium nitrate. The results of such an experiment are given in Table XIII and plotted in Fig. 6, showing the amounts of ammonia and nitrate formed in the soils of



Accumulation of  $\text{NH}_3$  and nitrate in soil *RC* by itself and after addition of ammonium sulphate.



Accumulation of  $\text{NH}_3$  and nitrate in soil *RC* by itself and after addition of  $\text{NaNO}_3$  (Table XIII, 1).



Accumulation of  $\text{NH}_3$  and nitrate in soil *RC*. Comparison of effects of  $\text{NaNO}_3$  and  $(\text{NH}_4)_2\text{SO}_4$ .

Fig. 6. Effect of additions of ammonium sulphate and sodium nitrate on the rate of accumulation of ammonia and nitrate in soils (Table XIII).

Fig. 4 in presence of ammonium sulphate or sodium nitrate. The limiting effect on the decomposition is well seen. The soil *RC* by itself behaves in the normal way, ammonia and nitrate accumulating on the tolued more rapidly than on the untreated soil. After addition of ammonium sulphate, however, the accumulation no longer goes on so quickly in the tolued soil, and the difference between the curves for this and the untreated soil becomes very small. Sodium nitrate also has an effect but not as great as that of ammonium sulphate. The untreated soil (which is poorer in ammonia and nitrate) behaves differently; neither the addition of ammonium sulphate nor of sodium nitrate has reduced the rate of decomposition. But even at the end of the period the total amount of ammonia and nitrate in this soil still remains below the quantity present in the tolued soils.

§ 40. In soil *OxL* a similar result is obtained. The rate of production of ammonia is at first much higher in the tolued than in the untreated soil. After addition of ammonium sulphate, however, the difference in the rates is considerably reduced, the tolued soil + ammonium sulphate having in six weeks gained little more than the untreated soil + ammonium sulphate. Addition of sodium nitrate appears to be without effect. After the first period of six weeks a drop is observed in the amounts of ammonia and nitrate in the tolued soil and the tolued soil + ammonium sulphate, but not in the other soils. This drop is unusual, and we have rarely observed it in our numerous experiments<sup>1</sup>; the usual course is for the ammonia and nitrate either to remain unaltered or to increase.

§ 41. Two causes may account for the limiting effect exerted by the added ammonium sulphate and sodium nitrate. We may suppose, as Bréal<sup>2</sup> did, that the production of ammonia still continues but a reverse action sets in as soon as the amount of ammonia and nitrate reaches a certain limit, and assimilation of ammonia then takes place. In the numerous cases where the amounts of ammonia

<sup>1</sup> We attribute it to loss of ammonia by volatilisation because we only get it where much ammonia is present and where there has been a distinct loss of water by volatilisation. Thus in the present instance the tolued soil and the tolued soil + ammonium sulphate contained initially 41.5 % and 43.6 % of water, whilst at the end they contained 37.4 % and 35.2 %, losses of 4.1 % and 8.4 % respectively. The amounts of ammonia present reached the unusually high figures of 134 and 277 parts per million (nitrification being considerably retarded) and the falling off from the usual straight line on the curve (*i.e.* the amount we suppose to be lost) to 37 and 49 parts per million.

<sup>2</sup> *Annales Agronomique*, 1896, 22, 449. Bréal had observed that the production of ammonia went on when the nitrification was suspended by treatment with antiseptics, but soon came to an end. He explained this by assuming assimilation.

TABLE XIII. Amounts of ammonia and nitrate produced in presence of ammonium sulphate and sodium nitrate respectively.

(1) Soil RC, containing 25 % moisture, 0.37 % N, 0.57 %  $\text{CaCO}_3$  and losing 11.05 % on ignition.

	NH <sub>3</sub> and nitrate present in—						NH <sub>3</sub> and nitrate produced		
	Untreated soil		Soil that received (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>		Soil that received NaNO <sub>3</sub>		without addition of (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> or NaNO <sub>3</sub>	in presence of (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	in presence of NaNO <sub>3</sub>
			as NH <sub>3</sub>	as NH <sub>3</sub> + nitrate	N as NH <sub>3</sub>	as NH <sub>3</sub> + nitrate			
	N as NH <sub>3</sub>	N as NH <sub>3</sub> + nitrate							
Untreated soil at start.....	5	113	88	210	5.5	220			
"    " after 43 days	3.5	139	3	248	4	244	26	38	24
"    "    " 92 "	7	156	4.5	263	5	263	43	53	43
Toluened soil at start.....	(10)	87	113	212	11	218			
"    " after 43 days	59	144	145	257	66	282	57	45	64
"    "    " 92 "	90	214	165	275	91	292	127	63	74
Heated soil at start.....	(30)	136	114	227	30	245			
"    " after 43 days	97	207	169	291	107	337	71	64	82
"    "    " 92 "	7	241	163	296	68	346	105	69	101



and nitrate remain constant we have further to suppose that the assimilation proceeds at the same rate as the ammonia production, so that only the excess over and above a certain quantity is assimilated. It is also necessary to assume an assimilation of nitrates on the same lines.

The simpler and more probable view is that ammonia production ceases in the soils as soon as a certain amount of ammonia and nitrate is present, the large quantity of ammonia and of soluble nitrates operating as a limiting factor and stopping ammonia production but not necessarily bacterial multiplication. The shape of the curves strongly supports this view, which is further in better accordance with the general nature of biochemical changes. We have also adduced evidence against the assimilation hypothesis in our earlier paper.

§ 42. Table XIV shows that the further increase in numbers on the reintroduction of the original flora into the partially sterilised soil is frequently accompanied by further increases in the amount of ammonia and nitrate produced, but the rule is by no means universal. Whenever the amount of ammonia is high and that of ammonia + nitrate is getting towards the limit, there is a tendency for the rule to be broken.

§ 43. *Partial sterilisation by heat.* The problem presented by heated soils is complicated by at least three disturbing factors. In the first instance heat effects a far more drastic reduction in the bacterial flora than toluene, so that the flora on a heated soil is much simpler than that on a tolued soil. Secondly, it causes a certain amount of decomposition of the organic matter, as is proved by the liberation of ammonia and the dark colour of the aqueous extract. This decomposition lightens the subsequent work of the bacteria with the result that the amount of ammonia and nitrate ultimately produced is much higher than when the partial sterilisation has been effected by antiseptics. Lastly, some of the decomposition products (we have not yet ascertained which) have a toxic action on bacteria so that multiplication does not go on as rapidly as in soils treated with toluene.

§ 44. We do not propose to discuss these effects in detail. Their operation is seen in Table XV where the results of some of our experiments are set out. In 1 the maximum numbers of bacteria are found in the soil that has been heated to 65°, the lowest temperature above that at which the detrimental organisms are destroyed and bacteria can begin to multiply. The numbers are lower in soil heated to 75° and a little lower in that heated to 85°, but very distinctly lower after

heating to 100°, where in fact they are little if any above those in the untreated soil. Similar results are shown by 2, indeed in all the cases so far examined we find maximum bacterial numbers in those soils that have been heated to the minimum temperature necessary to kill the detrimental organisms. Two causes seem to be at work. At this minimum temperature the extermination of the various species is less complete, and we have already seen (§ 26) that a mixed flora can occupy the ground more fully than a simpler one and so attain higher numbers. Also the toxic decomposition products are less in evidence. Soils heated to these minimum temperatures, in fact, closely resemble those treated with volatile antiseptics, since in both cases the secondary disturbances are at a minimum.

§ 45 But if the bacterial numbers are at a minimum in the soils heated to 100° the decomposition effected is at a maximum. There are only a few exceptions to this rule when the amounts of ammonia and nitrate are taken as the measure of the decomposition, and none when the amount of nitrogen assimilated by the plant is taken.

§ 46. The conclusion to which our experiments lead is that a relationship can generally be traced between the bacterial numbers and amount of decomposition in soils that have been heated to 55°—60° (this being the minimum necessary for killing the destructive organisms), just as it can in soils treated with volatile antiseptics; the same limitations also hold in both cases. But no such relationship exists in soils that have been heated to 100°.

Drying the soil has the same effect as heating to low temperatures (Tables VI and XV).

*The re-establishment of the original flora, and the introduction of the detrimental organisms.*

§ 47. The results of typical experiments on this subject are given in Table XIV. In (1), an arable soil was used containing initially much less ammonia and nitrate (8 parts) than the soil can stand (over 60 parts). In accordance with the general rule we find that the increase in bacterial numbers brought about by partial sterilisation is accompanied by an increase in the amount of ammonia and nitrate in spite of the accumulation of ammonia (see § 38): while the increase in numbers brought about by the addition of bacteria from the untreated soil leads to still further production of ammonia and nitrate. (The ammonia, it should be noted, disappears during the process.)

§ 48. The falling off in bacterial numbers in the infected soils is accompanied by a falling off in the production of ammonia and



TABLE XIV. *Bacterial numbers and amounts of ammonia and nitrate in partially sterilised soils reinfected with the bacterial flora of the untreated soils.*(1) Arable soil containing 17% water, 0.18% N, 3.16% CaCO<sub>3</sub> and losing 4.6% on ignition.

Mixture containing		Bacterial numbers, millions per gram of soil after				N as NH <sub>3</sub> , parts per million of dry soil after				N as NH <sub>3</sub> + nitrate, parts per million of dry soil after			
Untreated soil	Toluened soil	38 days	101 days	158 days	266 days	38 days	101 days	158 days	266 days	38 days	101 days	158 days	266 days
100	0	16	9	13	6	2	1	1	1	8	14	22	46
95	5	23	17	15	9	2	1	1	1	13	17	22	42
80	20	16	17	17	8	2	1	1	1	14	21	26	46
50	50	18	25	16	11	2	1	1	0	26	29	38	52
20	80	45	15	23	11	2	1	1	0	26	37	42	55
5	95	60	66	34	18	10	1	1	1	26	42	49	65
0.5	99.5	60	71	46	25	19	.5	2	1	26	43	50	62
0	100	43	41	43	18*	20	27	28	18*	27	34	34	50*

\* Infection apparently took place between the 158th and 266th day, as the nitrates, which had remained constant at 7 parts per million, rose during this period to 32 parts.



TABLE XIV—Continued.

(4) Soil SB, containing 16% moisture, 0.22% N, 0.63% CaCO<sub>3</sub>, and losing 6.0 c/o on ignition.

	Bacterial numbers, millions per gram of dry soil			N as NH <sub>3</sub> , parts per million of soil			N as NH <sub>3</sub> and nitrates, parts per million of soil		
	At start	After 21 days	After 113 days	At start	After 21 days	After 113 days	At start	After 21 days	After 113 days
Untreated soil									
Toluened soil *	9	36	19	7	3	4	93	122	145
" " + water extract of untreated soil	4	73	33	7	20	4	95	121	154
" " + 0.5 c/o of untreated soil (p. 179)	4	131	53	7	19	4	95	120	155
	4	122	70	7	23	4	95	116	151

\* Action incomplete, nitrifying organisms not being killed and nitrification only temporarily checked.

(5) Soil SB, containing 19% moisture.

Mixture containing		Bacterial numbers, millions per gram			N as NH <sub>3</sub> , parts per million			N as NH <sub>3</sub> and nitrate, parts per million of dry soil				
Untreated soil	Toluened soil	After 33 days	After 82 days	After 168 days	At start	After 33 days	After 82 days	After 168 days	At start	After 33 days	After 82 days	After 168 days
100	0	33	33	37	13	4	2	4	96	92	97	130
20	80	123	83	148	10	14	0	1	94	132	89	161
5	95	104	51	173	9	19	2	2	93	121	164	169
0.5	99.5	97	80	231	9	31	1	2	93	117	147	162
water extract	100	94	51	202	9	38	2	6	93	123	159	145
0	100*	103	90	—	9	43	2	—	93	134	118	—

\* Action incomplete, nitrifying organisms not being killed and nitrification only temporarily checked.

TABLE XIV—Continued.

6) Rich soil B, containing 30 % water. The air-dried soil contained 0.72 % N, 0.92 % CaCO<sub>3</sub>, and losing 25.0 % on ignition.

Mixture containing		Bacterial numbers, millions per gram				N as NH <sub>3</sub> , parts per million of dry soil				N as NH <sub>3</sub> and nitrate, parts per million of dry soil			
Untreated soil	Toluened Soil	After 15 days	After 69 days	After 154 days	After 15 days	After 69 days	After 154 days	After 15 days	After 154 days	After 15 days	After 154 days	Calculated amount* after 154 days	
100	0	101	83	20	22	8	13	96	178				
50	50	136	74	28	60	66	9	141	220			239	
20	80	267	171	122	75	55	4	160	272			276	
5	95	243	117	170	119	40	16	176	324			292	
0.5	99.5	233	255	74	98	63	205	162	270			298	
water extract	100	230	204	192	129	131	89	174	299				
0	100	152	225	202	137	149	173	205	260				

\* Calculated from the values for the untreated soil, and the tolued soil + water extract of untreated soil (i.e. tolued soil + bacteria of untreated soil, but with minimum accompaniment of detrimental organisms).

(7) Soil *OxL*, containing 46 % moisture, 0.63 % N, 1.9 % CaCO<sub>3</sub>, and losing 17 % on ignition.

		Bacterial numbers, millions per gram				N as NH <sub>3</sub> , parts per million of dry soil				N as NH <sub>3</sub> and nitrate, parts per million of dry soil			
		At start	After 15 days	After 113 days		At start	After 15 days	After 113 days	At start	After 15 days	After 113 days		
Untreated soil	.....	71	82	45		16	17	18	191			360	455
Toluened soil *	.....	146	245	185		35	99	99	225			381	465
" + water extract of untreated soil	.....	146	408	250		35	101	16	225			404	449
" + 0.5 % untreated soil (p. 179)	.....	146	287	222		35	107	10	225			398	†

\* Action incomplete, nitrifying organisms not being killed and nitrification only temporarily checked. † Lost.

TABLE XV. *Bacterial numbers, and amounts of ammonia and nitrate in soils partially sterilised by heat\* and by drying.*

1. Arable soil as before containing 14 % water.

Tempera- ture to which soil is heated	Bacterial numbers, millions per gram						N as N H <sub>3</sub> , parts per million						N as N H <sub>3</sub> and nitrate, parts per million																
	Soon after starting		After 15 days		After 120 days		After 15 days		After 120 days		After 178 days		After 205 days		After 319 days		At start		After 15 days		After 120 days		After 178 days		After 205 days		After 319 days		
	After 7 days	After 15 days	After 15 days	After 15 days	After 120 days	After 120 days	After 15 days	After 15 days	After 120 days	After 120 days	After 178 days	After 178 days	After 205 days	After 205 days	After 319 days	After 319 days	At start	At start	At start	At start	At start	At start	At start	At start	At start	At start	At start	At start	
—	11	9	9	4	—	—	2	1	1	1	—	—	—	—	—	—	34	34	39	39	—	—	—	—	—	—	—	—	—
45°	13	11	11	9	12	12	2	2	2	2	10†	2	—	—	—	—	—	35	49	57	57	53	—	—	—	—	—	—	—
52°	15	11	11	9	23	23	3	11	1	1	18†	3	—	—	—	—	—	40	57	75	75	65	73	—	—	—	—	—	—
55°	—	—	—	5	3	13	73	—	15	14	6	4	3	—	—	—	—	42	69	72	72	69	63	63	63	63	63	63	63
65°	13	21	37	45	60	60	7	16	21	24	33	30	—	—	—	—	—	42	65	59	59	66	63	63	63	63	63	63	63
75°	2	12	32	26	45	45	—	22	33	36	41	40	—	—	—	—	—	49	64	64	64	75	76	76	76	76	76	76	76
85°	4	10	37	14	44	44	6	23	32	46	36	42	—	—	—	—	—	47	61	75	75	63	78	78	78	78	78	78	78
100°	1.4	3	18	8	14	14	6	22	44	46	55	51	—	—	—	—	—	49	69	75	75	79	79	81	81	81	81	81	81

\* The soils were heated for 3 hours in the bottles in which they were stored.

† We cannot account for these abnormally high ammonia results, which would usually only be about 2 parts per million. The values for ammonia and nitrate are correspondingly high.

2. Arable soil as before containing 13 % water.

Tempera- ture to which soil is heated	Bacterial numbers, millions per gram						N as NH <sub>3</sub> , parts per million						N as NH <sub>3</sub> and nitrate, parts per million																
	At start		After 7 days		After 68 days		After 142 days		At start		After 7 days		After 21 days		After 68 days		After 142 days		At start		After 7 days		After 21 days		After 68 days		After 142 days		
	After 7 days	After 7 days	After 7 days	After 7 days	After 68 days	After 68 days	After 142 days	After 142 days	After 7 days	After 7 days	After 7 days	After 7 days	After 21 days	After 21 days	After 68 days	After 68 days	After 142 days	After 142 days	At start	At start	At start	At start	After 7 days	After 7 days	After 21 days	After 21 days	After 68 days	After 68 days	After 142 days
—	11	10	12	11	4	4	0	2	1	1	1	1	1	1	1	1	1	1	9	9	10	10	9	9	13	13	19		
40°	7	9	10	8	3	3	0	1	3	3	3	3	3	3	3	3	3	3	9	9	11	11	15	15	14	14	32		
56°	2	14	16	38	48	48	2	7	9	9	23	23	2	2	2	2	2	2	10	10	14	14	17	17	37	37	45		
70°	2	17	11	24	27	27	3	8	11	16	16	20	20	20	20	20	20	20	12	12	16	16	23	23	22	22	27		
100°	0.1	17	22	10	10	10	3	6	14	19	33	33	33	33	33	33	33	33	14	14	16	16	23	23	25	25	40		

TABLE XV—Continued.

## 3. Various richer soils.

*MT* containing 16  $\frac{1}{2}$  water, 0.26  $\frac{1}{2}$  N, 1.0  $\frac{1}{2}$  CaCO<sub>3</sub> and losing 7.8  $\frac{1}{2}$  on ignition.

	Soil <i>MT</i>						Soil <i>RC</i>					
	N as NH <sub>3</sub> , parts per million of dry soil			N as NH <sub>3</sub> and nitrate, parts per million			N as NH <sub>3</sub> , parts per million of dry soil			N as NH <sub>3</sub> and nitrate parts per million		
	At start	After 32 days	After 114 days	At start	After 32 days	After 114 days	At start	After 23 days	After 112 days	At start	After 23 days	After 112 days
Untreated soil	4	8	9	50	63	66	24	12	10	188	178	241
Treated with toluene	5	44	35	49	100	93	39	103	132	196	255	298
Heated to 55°	10	5	6	62	67	78	49	93	9	185	292	270
" " 100°	19	53	78	78	119	152	52	160	184	217	325	393

	Garden soil						Soil <i>KH</i>					
	N as NH <sub>3</sub> , parts per million of dry soil			N as NH <sub>3</sub> and nitrate, parts per million			N as NH <sub>3</sub> , parts per million of dry soil			N as NH <sub>3</sub> and nitrate, parts per million		
	At start	After 124 days	At start	After 124 days	At start	After 124 days	At start	After 63 days	At start	After 63 days	At start	After 63 days
Untreated soil	6	8	50	90	2	3	2	45	26	29	26	29
Treated with toluene	15	9	56	129	8	45	8	41	31	71	31	71
Heated to 55°	23	23	59	128	10	41	10	41	34	66	34	66
" " 100°	22	87	69	165	11	45	11	45	34	66	34	66

TABLE XV—*Continued.*

## 4. Effect of drying.

(1) Soil R' containing 24 c/o water, composition when air-dried given above.

	Bacterial numbers, millions per gram			N as NH <sub>3</sub> , parts per million			N as NH <sub>3</sub> and nitrate, parts per million		
	At start	After 26 days	After 42 days	At start	After 26 days	After 42 days	At start	After 26 days	After 42 days
Untreated	27	28	39	5	2	3	91	105	105
Dried*	7	37	59	15	3	2	96	139	141
									Gain in 42 days
									14
									45
(2) Soil K11									
Untreated	5	5	8	0.5	2	1	57	66	65
Dried*	4	8	10	14	18	24	70	74	85
									8
									15

\* For 10 days in a hot chamber at 35°—38°.

nitrate. But we know that this would have happened in any case as the result of the accumulation of ammonia and nitrate already formed and we cannot therefore conclude that it is connected with the drop in bacterial population. Indeed the decomposition has already gone so far before the reduction in bacterial numbers sets in—two-thirds of the final quantity of ammonia and nitrate being already formed, and the remaining one-third being on its way<sup>1</sup>—that but little work remains to be done.

§ 49. Entirely similar results were obtained in other experiments with arable soils, and in some of the experiments with richer soils. In (6) for instance (same Table), the bacterial numbers are at first much higher in the infected soils than in the tolunened soil and the amount of decomposition subsequently becomes higher. But even after five months, when the process is well on to completion, there is no evidence that the fall in bacterial numbers has adversely affected the rate of accumulation of ammonia and nitrate, for the quantities actually found correspond fairly well with the numbers calculated on the assumption that the untreated soil and the tolunened soil + water extract of untreated soil (*i.e.* tolunened soil + bacteria of untreated soil but with minimum accompaniment of detrimental organisms) are inert to one another.

On the other hand, instances have also been observed where the drop in bacterial numbers is accompanied by a marked falling off in the rate of decomposition<sup>2</sup>.

§ 50. The other experiments recorded in the Table illustrate the case where a large amount of ammonia, or of ammonia and nitrate, has already accumulated, and where fresh accumulation is no more rapid than in the untreated soils. No relationship therefore exists between bacterial numbers and rate of decomposition.

§ 51. The conclusion to be drawn from these experiments is that the increased bacterial numbers resulting from the introduction of the original flora into the partially sterilised soil leads to an increased production of ammonia and nitrate unless too large a quantity of these substances is already present. But the subsequent depression in bacterial numbers consequent on the development of the detrimental organisms is generally (though not always) without effect on the rate of decomposition, apparently because it does not set in until too late.

<sup>1</sup> We have shown in our previous paper that the amount of unstable intermediate products as well as of ammonia is increased by partial sterilisation.

<sup>2</sup> *E.g.* this *Journal*, 1912, 5, 98.



*The effect of varying temperatures of incubation on the changes taking place in partially sterilised soils.*

§ 52. The experiments dealt with in the preceding sections were mainly carried out at the ordinary laboratory temperature. A series was now undertaken at higher temperatures, the bottles of soil being stored in incubators maintained respectively at 20°, 30°, 40° and 50°. The results obtained are set out in Table XVI.

We have already dealt with the bacteriological data and need now only point out that an increase in temperature from 10° to 20° fails to increase the bacterial numbers in the untreated soil, but it does increase them in the tolued soil, a fact that has been fully discussed in §§ 4 and 5. The bacterial numbers attain a maximum in the soil stored at 20° though they are still high at 30°, they fall off rapidly at 40° and still more completely at 50°. At these two higher temperatures there is no difference in bacterial numbers in the tolued and the untreated soils. The curves obtained in studying one of the soils are given in Fig. 7.

The rate of accumulation of ammonia and nitrate is connected with the bacterial numbers only in the tolued soils kept at 5°—12° and at 20°. The bacterial numbers increase at 20° in comparison with those at the lower temperature and so also does the rate of accumulation of ammonia and nitrate until the usual falling off sets in, when the relationship ceases to exist. In all other cases there is a complete absence of any sort of relationship. The rate of accumulation of ammonia and nitrate is greater at 20° than at 5°—12°, although the same limit may be reached in both cases; it is also generally greater in the tolued soil than in the untreated soil. At 30° it is still greater and in all but one instance it proceeds much further than at the lower temperatures; it becomes rapid at 40° and still more so at 50° and proceeds at each temperature to a correspondingly higher extent. We get, therefore, a series of curves in which the rate of accumulation of ammonia and nitrate successively increases with increasing temperature and in which also the extent of the accumulation also increases—to a small extent—at first but very much afterwards. This is wholly unlike the series obtained for bacterial numbers, excepting, as above mentioned, on the tolued soils at about 10° and 20°.

§ 53. Thus we have another exception to the rule that bacterial numbers are connected with the rate of accumulation of ammonia and

nitrites. But it is not necessarily a significant exception, for the decomposition at the higher temperatures may be a chemical process wholly unconnected with bacteria<sup>1</sup>. For the present we prefer to leave this

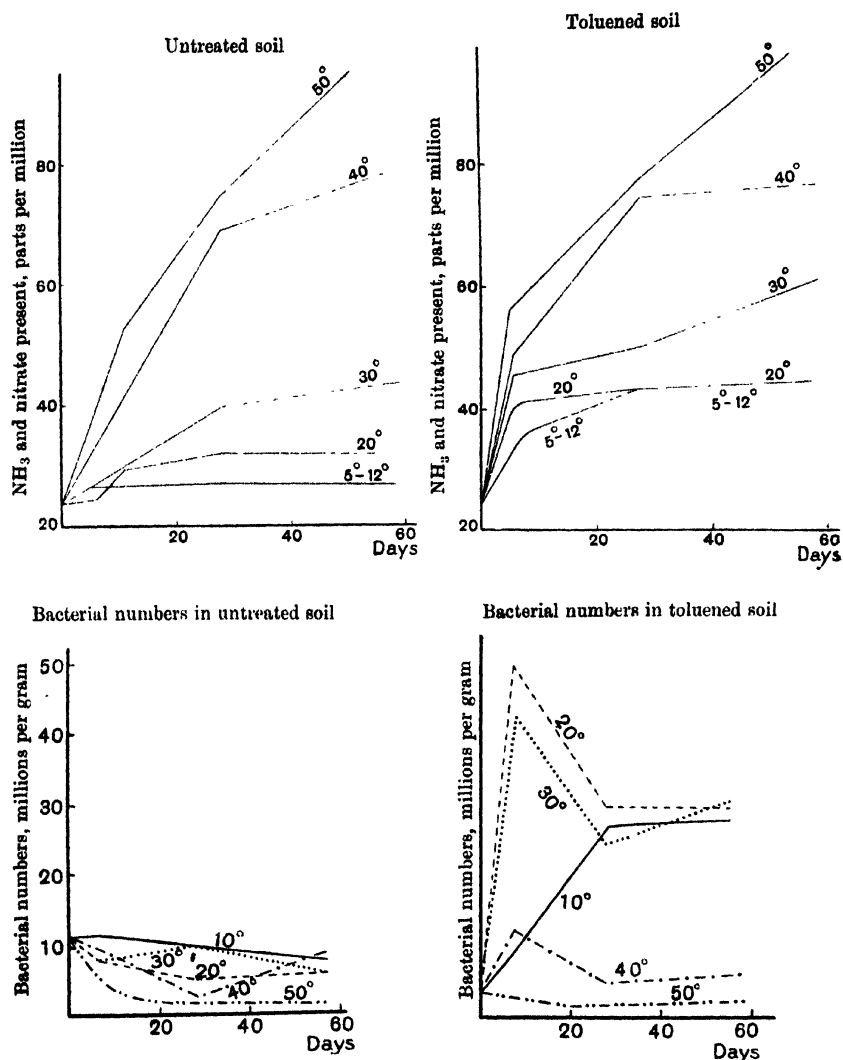


Fig. 7. Effect of varying temperatures of storage on the rate of accumulation of ammonia and nitrate in soils (Table XVI, arable soil).

<sup>1</sup> It will be noticed that ammonia accumulates in the soils maintained at 40° and over, showing that the nitrifying organisms no longer work much, if at all.

TABLE XVI. *Effect of varying temperatures on the changes taking place in partially sterilised soils.*

1. Arable soil as before containing 14 % water.

	Bacterial numbers, millions per gram			N as $\text{NH}_3$ , parts per million			N as $\text{NH}_3$ and nitrate, etc., parts per million		
	After 5 days	After 27 days	After 58 days	After 5 days	After 27 days	After 58 days	After 5 days	After 27 days	After 58 days
Untreated soil kept at									
	11	9	7	1	2	1	27	27	27
	8	5	6	2	2	1	24	32	31
	8	9	6	2	1	3	29	41	44
	9	2	8	2	13	36	43	70	79
50°	4	1	1	23	42	80	53	75	117
Toluened soil kept at									
	8	27	28	13	19	23	32	44	45
	50	30	30	19	18	7	39	44	45
	43	24	31	23	15	3	46	50	62
	12	4	6	27	47	49	48	74	76
50°	2	1	1	35	56	72	56	73	102



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question undecided until other investigations now in hand of the chemical processes are further advanced. It may also be doubted whether our method of counting is applicable to the soils stored at higher temperatures. Gelatine plates kept at 20° may not provide suitable conditions for the development of such thermophilic organisms as may be active in a soil maintained at 40°.

§ 54. It is possible to treat the curves mathematically after the methods adopted by physical chemists, but the data are hardly fine enough since the error of the determinations becomes rather considerable at the higher temperatures. There is an unknown loss of ammonia (see footnote, p. 197) and there also appears to be a production of reducible substances not present in appreciable amounts in ordinary soils, but giving rise to ammonia on reduction with the zinc-copper couple, and therefore appearing in the analytical results as nitrates.

### *The effect of additional food on bacterial numbers and the rate of decomposition.*

§ 55. It has already been pointed out that all the antiseptics used have some direct action in the soil which is shown in our experiments by a liberation of ammonia. We therefore have to consider the possibility that other substances may be set free capable of acting as food for bacteria, and it becomes necessary to ascertain how added food-stuffs affect the bacterial numbers and the amounts of decomposition. We have limited ourselves to three substances: sugar, hay dust and peptone.

§ 56. All of these substances cause the bacterial numbers to go up rapidly for a time, but there is no corresponding increase in the amounts of ammonia and nitrate such as is obtained on partial sterilisation. Sugar, indeed, causes a marked loss of nitrate and no increase in ammonia; hay dust has a similar but less marked action, whilst peptone fails to increase the stock of ammonia and nitrate even by the full amount of the nitrogen it contains. Supposing therefore any foodstuff to be liberated by the antiseptics used we should expect it to increase the bacterial numbers but not necessarily the ammonia and nitrate. This would happen only if the liberated substances themselves gave rise to the extra ammonia and nitrate, i.e. if they were easily decomposable after contact with antiseptic vapours, but not before.

*The limitations of the methods for counting bacteria.*

§ 57. Three facts stand out prominently on reviewing the whole of the data obtained at ordinary temperatures (*i.e.* not above 20°):

(1) An increase in bacterial numbers effected by partial sterilisation commonly causes an increase in the amounts of ammonia and nitrate.

(2) But if a large amount of ammonia or of ammonia and nitrate is already present in the soil the increased bacterial numbers do not necessarily bring about more production of these substances.

(3) Whenever an increase in the rate of production of ammonia and nitrate is obtained there is always an increase in bacterial numbers<sup>1</sup>.

(2) and (3) may also be expressed thus: bacterial multiplication may take place without an increased rate of production of ammonia and nitrate, but an increased rate of production of ammonia and nitrate does not occur without bacterial multiplication.

§ 58. In some of our experiments the relation between bacterial numbers and amounts of ammonia and nitrate is fairly close (*e.g.* Fig. 4, Case 1), but more usually this is not so. From what we know of the limitations of the counting method indeed, we should hardly expect it to be otherwise. Some of the soil bacteria are more vigorous ammonia producers than others, some are not even active but only occur as spores. Yet all these are grouped together without any distinction. Further, the method does not even give the actual total but misses altogether those organisms that fail to develop on the plates employed; there is, in fact, no method for estimating the total number of bacteria in the soil.

§ 59. The question therefore arises, how far do the numbers revealed by any particular plate possess any significance? The data for a complete answer do not exist, but there is considerable evidence that on one and the same soil under strictly comparable conditions the fluctuations in the numbers growing on gelatine plates afford a satisfactory index of the fluctuations in the total numbers of decomposition bacteria. When therefore we find that partial sterilisation has increased the bacterial numbers revealed by our plates from 20 to 40 millions per gram we do not imply that the true totals have doubled under the treatment, but that they have increased; the exact amount of increase we cannot as yet specify. Further, we only institute the comparison where the conditions are otherwise identical; the untreated

<sup>1</sup> In the course of four years we have only found one exception. The results obtained at 30°, 40° and 50° are expressly excluded here.

TABLE XVII. *Effect of added organic matter on the bacterial numbers and rate of decomposition in untreated and partially sterilised soils.*A. Arable soil containing 15% moisture, 0.18% N, 0.37% CaCO<sub>3</sub> and losing 7.6% on ignition.

	Bacterial numbers, millions per gram				N as NH <sub>3</sub> , parts per million of soil				N as NH <sub>3</sub> and nitrate, parts per million of soil			
	After 6 days	After 62 days	After 101 days	After 215 days	After 6 days	After 62 days	After 101 days	After 215 days	After 6 days	After 62 days	After 101 days	After 215 days
Untreated soil alone	13	7	11	12	4	2	4	1	(57)	71	85	98
" + 0.25% sugar	129	97	32	26	2	3	2	6	5	24	37	71
" + 0.1% peptone	148	11	21	8	60	45	28	31	117	172	177	231
" + hay infusion	19	7	9	7	3	2	4	5	56	85	91	106
Toluened soil alone	19	6	27	23	16	31	30	54	58	79	80	101
" + 0.25% sugar	19	11	33	28	5	36	35	59	16	42	42	63
" + 0.1% peptone	14	19+	44	52	41	194	120	158	89	227+	169	204
" + hay infusion	15	8	22	24	10	32	11	47	54	77	95	95
Toluened soil alone	118	45	48	lost	8	31	26	37	56	79	84	104
" + 0.25% sugar	156	133	134	77	2	32	28	31	7	39	46	67
" + 5% un. treated soil	125	102	102	58	50	169	113	116	97	157	173	210
" + hay infusion	114	66	61	17	10	29	27	25	56	82	89	94
Toluened soil alone	84	25	106	lost	13	12	4	0	66	89	103	123
" + 0.25% sugar	178	100	163	59	4	12	12	3	12	57	74	92
" + 0.1% peptone	82	80	77	55	84	54	58	40	144	192	212	243
soil untreated	130	75	51	13	35	9	10	6	93	94	110	118
At beginning of experiment	Untreated soil	8					4				57	
" " "	Toluened soil	5					6				54	

\* Supplying 160 parts N per million of soil.

+ A double dose of peptone had inadvertently been added to this soil.

TABLE XVII—Continued.

B. Arable soil containing 15% moisture, 0.18% N, 3.16% CaCO<sub>3</sub> and losing 4.6% on ignition. organic matter added = 0.5% ground hay containing 1.43% N and adding 71.5 parts of N per million of soil.

	Bacterial numbers, millions per gram		N as NH <sub>4</sub> , parts per million		N as NH <sub>4</sub> and nitrate, parts per million	
	At start*	After 7 days	After 74 days	At start	After 7 days	After 74 days
Untreated soil, no hay	7	1	12	3	1	27
" " + hay	7	94	62	3	4	12
Toluened soil, no hay	31	41	38	25	4	58
" " + hay	31	175	136	25	10	20
Toluened soil + water extract of untreated soil, no hay	50	—	56	2	2	67
" " " " + hay	50	311	152	2	3	45
Toluened soil + 0.5% untreated soil, no hay	66	43	65	3	2	65
" " " " + hay	66	227	101	3	2	50
Toluened soil + 5% untreated soil, no hay	57	58	47	2	3	66
" " " " + hay	57	255	83	2	3	47
Toluened soil + 50% untreated soil, no hay	25	—	53	2	2	49
" " " " + hay	25	86	66	2	4	27

\* I.e. when hay was added; the soils had been treated 5½ months previously in order that the initial changes in the soil itself might be finished before the hay was put in.



and partially sterilised soils were initially the same, the water content, temperature and aeration conditions are as nearly as possible identical; no attempt is made to compare one soil with another under different conditions. With these limitations the method gives valuable results and can continue to be used till a better is devised.

Other media are in use besides gelatine, but so far as they have been tried they give the same kind of results, indicating fluctuations in the same direction as gelatine although the actual figures are different.

§ 60. Perhaps the most striking proof of the validity of the gelatine plate method as we use it is that it leads to precisely the same conclusions as methods of a wholly different character. For example, the gelatine plate method shows that the numbers of bacteria increase after partial sterilisation. We have seen that special tests of an entirely different nature show an increase of denitrifying organisms and of organisms causing loss of nitrogen after partial sterilisation (foot-note, p. 178); in our previous paper we showed that the rate of decomposition of peptone solution (in Remy's method) also increases. We have used these peptone solutions in testing some of the deductions drawn from the gelatine plate experiments, and have invariably found that both methods gave the same results. Two instances only need be given:

(1) From the gelatine plate counts we concluded that the effect of partial sterilisation was to improve the soil as a medium for bacterial growth and not to improve the bacteria as decomposition agents (§§ 24 and 25). When *small* inoculations of soil are made into peptone solutions so that the effect shall be that of bacteria and not of soil, the partially sterilised soil is no better than the untreated. But when *large* inoculations are used, so that the effects of the detrimental organisms can come into play, the partially sterilised soil is distinctly better than the untreated. The amounts of ammonia in milligrams produced from a one per cent. peptone solution were:

		Hours			
		After 24	32	44	90
<i>Small inoculation</i> (effects of bacteria only)	Untreated soil . . .	0.5	7.3	8.7	21.0
	Toluened soil . . . .	0.5	7.5	8.5	20.9
<i>Large inoculation</i> (effects of bacteria and of detrimental organisms)	Untreated soil . . .	4.9	15.2	21.6	33.5
	Toluened soil . . . .	6.2	18.0	23.6	34.5

(2) From the gelatine plate experiments we concluded that the detrimental factor was not associated with the water extract but with

the soil itself. A *clear, filtered* water extract of the untreated soil was found to be more effective in decomposing peptone than a similar extract of *toluened* soil, but a *turbid* extract was distinctly less effective. The amounts of ammonia in milligrams produced from one per cent. peptone were :

	After	Hours	
		65	90
Suspension of untreated soil, muddy		20.4	28.4
„      toluened      „      „		24.2	34.7
„      untreated soil filtered through cotton wool, turbid		19.1	25.1
„      toluened      „      „      „      „      „		20.1	30.0
„      untreated soil filtered through paper, clear		5.0	11.2
„      toluened      „      „      „      „		5.9	8.7

Thus in both cases the experiments lead to the same conclusion although the methods are fundamentally different. On the whole we prefer the gelatine plates to the culture solutions, but we use the culture solution methods to check the results yielded by the gelatine plates.

#### *Summary and Conclusions.*

The conclusions reached in our previous paper have been confirmed and extended. Fresh evidence is adduced that bacteria are not the only inhabitants of the soil, but that another group of organisms occurs, detrimental to bacteria, multiplying more slowly under soil conditions and possessing lower power of resistance to heat and to antiseptics.

In consequence of the presence of these detrimental organisms the number of bacteria present in the soil at any time is not a simple function of the temperature, moisture content and other conditions of the soil. It may, indeed, show no sort of connection with them; thus rise of temperature is found to be ineffective in increasing the bacteria in the soil; increase in moisture content has also proved without action. The number of bacteria depends on the difference in activity of the bacteria and the detrimental organisms.

But when soil has been partially sterilised the detrimental organisms are killed and the bacteria alone are left. It is then found that increase in temperature (up to a certain point) favours bacterial multiplication and causes the numbers to rise. Variations in moisture content also produce the normal results on partially sterilised, but not on untreated soils.

The detrimental organisms are killed by any antiseptic vapour or by heating the soil to 55°–60° C.: they suffer considerably when the

soil is maintained at lower temperatures (40° C.) for a sufficient length of time. Cooling to low temperatures also depresses them although it fails to kill them.

The completeness of the process can be accurately gauged by the extent to which the bacteria suffer. Whenever the treatment is sufficiently drastic to kill the nitrifying organisms and to reduce considerably the numbers of the other bacteria (as shown by the counts on gelatine plates) it also kills the detrimental organisms. If the soil conditions are now made normal, and the antiseptic is completely removed, rapid increase is observed in the bacterial numbers and the rate of production of ammonia. A temporary or partial suppression of the factor is, however, possible without extermination of the nitrifying organisms.

Once the detrimental organisms are killed the only way of introducing them again is to add some of the untreated soil. But the extent of the transmission is apt to be erratic, being sometimes more and sometimes less complete than at others; occasionally the infection fails altogether. We have not yet learned the precise conditions governing the transmission.

Provisionally we identify the detrimental organisms with the active protozoa of the soil, but as the zoological survey is yet incomplete we do not commit ourselves to any particular organism or set of organisms or to any rigid and exclusive definition of the term protozoa.

The increase in bacterial numbers following after partial sterilisation by volatile antiseptics is accompanied by an increase in the rate of ammonia production until a certain amount of ammonia or of ammonia and nitrate has accumulated, when the rate falls. Thus two cases arise: (1) when only small amounts of ammonia and nitrate are present there is a relationship between bacterial numbers and the rate of ammonia production, (2) when large amounts of ammonia or of ammonia and nitrate are present there is no relationship. The limit varies with the composition and condition of the soil.

Complications are introduced when the soil has been partially sterilised by heat, because heat effects an obvious decomposition of the organic matter, thus changing the soil as a medium for the growth of micro-organisms. The bacterial flora is also very considerably simplified through the extermination of some of the varieties. These effects become more and more pronounced as the temperature increases, and their tendency is to reduce the numbers of bacteria. We find maximum bacterial numbers in soils that have been heated to the minimum

temperature necessary to kill the detrimental organisms (about 60°). Both bacterial numbers and the rate of decomposition in such soils approximate to those obtaining in soils treated with volatile anti-septics, and the above-mentioned relationships between these quantities also hold.

Although bacterial numbers are at a minimum in soils heated to 100° the decomposition effected is at a maximum.

With this exception it is generally true that bacterial multiplication may go on without increasing the rate of production of ammonia, but an increase in the rate of production of ammonia does not take place without bacterial multiplication.

The increase in bacterial numbers brought about by addition of bacteria from the untreated soil into partially sterilised soil leads to still further production of ammonia and nitrate unless too large a quantity of these substances is already present. But the subsequent depression in bacterial numbers consequent on the development of the detrimental organisms is generally (though not always) without effect on the rate of decomposition, apparently because it does not set in until too late.

## THE MICRO-FLORA OF STILTON CHEESE.

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WHEN well made a Stilton is usually admitted to be the finest of the English varieties of cheese, but like all choice dairy products it is exceedingly difficult to manufacture of uniformly good quality. Great uncertainty exists, even amongst the most experienced makers, in regard to the factors which govern the excellence of flavour and texture in this kind of cheese: the high moisture content favours the rapid growth of harmful as well as useful organisms and "off flavour," objectionable taints, and irregular consistence due to the activity of the former class are common.

Some five years ago the authors began the study of the micro-flora of Stilton cheese, in the hope that a more complete knowledge of the organisms present would assist in the elucidation of the ripening process, and be a step towards placing the manufacture of cheese of fine quality on a sound basis.

In the first instance cheeses made at the British Dairy Institute under the supervision of Mr Miles Benson were investigated; later examples from the best dairies of the Melton Mowbray district were chosen for examination. Our work has been directed towards the determination of the kinds of bacteria and fungi present in normally ripened cheeses: at the same time we have endeavoured to estimate the numbers of organisms in cheeses at different periods of ripening. The investigation of both these problems has proved of great complexity, and much remains to be done.

We have, however, thought it opportune to give an account of the work already accomplished, for in regard to the normal aerobic species of bacteria and fungi which are found in this variety of cheese we consider that the investigation is fairly complete. We have not attempted to deal with the parts which the several kinds of organisms play in the development of aroma, flavour, and texture of the cheese;

these problems, as well as the search for anaerobic organisms, must be left for future study.

Attention was first paid to a determination of the number of organisms in the cheese at various periods, from 24 hours old, up to complete ripeness, 80—100 days later.

Cylindrical pieces were withdrawn with a sterilised cheese-borer, driven into the cheese to approximately the same depth each time: one gram of the extracted core was rapidly weighed, and then rubbed up in water in a small sterilised glass mortar.

This was ultimately diluted to 1 in 1,000,000, and 1 c.c. of the dilution was added to a tube of the medium used for plating out. It was found that dilution to 1 in 100,000 was insufficient to allow of precise counting of the colonies, especially where very young cheeses were under examination.

Three media were used, namely lactose-gelatine (100 grams gelatine and 20 grams lactose per litre of medium, acidity 10+), a similarly acid lactose agar (1.5 % agar) and a neutral litmus lactose-gelatine.

After incubation of the gelatine plates at 22 deg. C. and the agar at 35 deg. C. for two days, the colonies were counted.

The distribution of the organisms in the cheese was studied by cutting thin slices of the latter with a razor and staining with eosin-methylene blue or thionine: examinations were also made of cover-glass impressions of the broken curd: both methods revealed an uneven grouping of organisms. Single isolated bacteria, mainly rods, occur fairly evenly distributed throughout the cheese, but the majority are found in irregularly arranged colonies of very variable size. On account of this irregularity in the disposition of the organisms, it is not possible to obtain strictly comparable samples from day to day from the same cheese, without taking a large number of separate borings on each occasion, a proceeding which would render the cheese useless for future investigation.

The results of our work indicate that so far as an estimation of the number is concerned, a gram of cheese taken from a single boring is too small a portion to be regarded as completely representative of the bulk of the core withdrawn. Moreover the numbers obtained differ with different media used for plating out: some grow better on one substance than on another, the numbers being considerably less on agar than on either of the gelatines employed. As indicated later, the presence of one kind of organisms more or less entirely prevents

the appearance of another species on the same plate. Extended trials have shown that a complete and precise estimate of the numbers of organisms in a cheese of the type of Stilton with its very moist and loosely packed curd cannot be attained by small samples obtained from single borings. However, repeated examination during the last four or five seasons of portions of single borings removed from the cheeses at short intervals has enabled us to obtain a general view of the rise and fall in the numbers of organisms and the changes which occur in the micro-flora as ripening proceeds.

In the first 48 hours there is an extraordinary development of organisms from 1000 to 3000 million per gram being frequently found, more than 90 % of which are cocci or short rods capable of producing lactic acid in milk. The numbers reach a maximum within four days, and decline slowly afterwards up to the time of complete ripeness, when the cheese contains from 50 to 100 millions per gram, mostly moulds and yeasts; concurrent with the diminution of bacteria there is a gradual rise in the development of fungi.

As mentioned later, a considerable number of species of bacteria and fungi are found in Stilton cheese, the greatest variety being found on plates from young specimens before the acidity has reached its maximum, and in the later periods of ripening when the acidity is lowest and open spaces due to shrinkage have begun to form: occasional moulds are met with throughout the whole period of ripening, but they make their appearance in increasing numbers when the cheese is 30—40 days old.

The great variety of organisms we have isolated may be classified into two groups, namely :—

(1) Bacteria and fungi, which from their constant occurrence in Stilton cheeses whenever and wherever they are made, may be regarded as normal constituents of the micro-flora, and concerned more or less directly with the ripening process of a good cheese.

(2) A miscellaneous group whose presence is accidental and whose influence is either harmless or detrimental to the quality.

*Normal Bacteria.* Three forms of bacteria are invariably present, namely, a coccus, a rod-shaped organism belonging to the lactic acid group, and a species of *Tyrophrix*.

1. *Coccus A* is a small roundish-oval organism, 0.4—0.5  $\mu$  in diameter (Fig. 1). It usually occurs in pairs, but occasionally may be found in short chains of three and four together. It grows slowly on solid media in the form of minute white colonies beneath the surface.

In milk it produces a dense uniform curd without gas bubbles or separation of whey. It curdles milk slowly, taking three days at 22 deg. C. to produce a solid curd.

We consider this typical *Streptococcus lacticus*. It is abundant in all cheeses we have examined.

A larger form is found in some cheeses with oval cells somewhat elongated— $1.1\ \mu$  to  $1.3\ \mu$  long. Just before or at the time of division, the organism resembles a short rod. In milk and in cheese 24 to 48 hours old it is found in well-developed chains, consisting of 6 to 12 or more cells. In cheeses a few days old, however, and on gelatine and agar media, the chain form is lost, the organism then appearing chiefly in pairs (Figs. 2 and 3). The colonies are white and very small— $\frac{1}{2}$  mm. or less in diameter: they grow beneath the surface of the medium. Milk is curdled by this bacterium in 24 hours or less when kept at 22 deg. C., the curd produced being dense and uniform, without gas bubbles or separation of whey.

The organism is a form of *Streptococcus lacticus*, and appears to be identical with the coccus present in most of the commercial starters so commonly used by butter makers and some makers of cheese: Stiltons containing it are of fair mild flavour, but too dry and acid. Possibly vigorous acid formers like this species may be useful in repressing the objectionable organisms found in dirty dairies, or where cheese is manufactured from doubtfully clean milk: we feel convinced, however, that they should find no place in a Stilton dairy where cheeses of the finest flavour and texture are desired.

2. *Bacterium A*. Another common characteristic bacterium in Stilton cheese is a short stumpy rod,  $2-4\ \mu$  long and  $0.7-0.8\ \mu$  thick (Fig. 4): sometimes it occurs in the form of slightly oval cells, and then resembles the large *Streptococcus* referred to above.

The colonies on the surface of the media are circular, from 1—2 mm. in diameter, white, moist and raised a little above the surface; in the substratum they are smaller and either round or spindle-shaped; old colonies become yellowish.

The organism is Gram positive and non-motile.

It curdles milk slowly, taking 6—8 days at 22 deg. C. to produce a dense curd, which very closely resembles that thrown down by *Streptococcus lacticus*; no gas bubbles appear, and there is little or no separation of whey.

When grown in broth it lengthens to 4 or  $4.5\ \mu$ , but preserves its somewhat oval shape: it appears to be a variety of *Bs. acidi lactici*



of Hueppe, but unlike the latter it does not ferment glucose nor lactose: some forms of it give a slight Voges and Proskauer reaction, but none produce indole.

In fully ripe cheeses lactic acid organisms are comparatively few in number; many die off altogether in the ripening process, and those which remain possess diminished vitality and are only able to acidify milk very slowly.

3. A species of *Tyrothrix* is invariably present in Stilton cheeses. It is found in all stages of ripening after the third or fourth day, but is never abundant.

It is the chief and usually the only organism which appears on plates inoculated with a "dilution" of the cheese heated to boiling point. The organism is rod-shaped, and feebly motile in young cultures. The individual cells from agar colonies are 6—12  $\mu$  long, 1—1.25  $\mu$  broad. In milk they are longer and thinner and often united in the form of tangled threads, some of which may reach a length of 150  $\mu$  (Fig. 5).

It forms oval spores, which measure 2  $\mu \times$  1  $\mu$ .

Gelatine is liquefied rapidly by it, and milk is rendered alkaline and coagulated, the curd being soon digested: no gas is produced in glucose, saccharose, or lactose bouillon.

The surface colonies on agar are white, round, smooth and moist at first; later they spread over large areas and become wrinkled and dry, the margins of such colonies being fimbriated. Beneath the surface of the medium the colonies remain small and are more or less granular with characteristic mycelioid or floccose margins.

The organism is closely allied to, if not identical with, Duclaux's *Tyrothrix tenuis*.

4. *Miscellaneous Bacteria*. In addition to the four species of bacteria already mentioned, which we consider from their physiological activities and constant presence are directly concerned with the ripening of Stilton cheese, there are many others of accidental occurrence. In the latter class we include *Bacterium coli*, *B. lactis aerogenes*, and many chromogenic species of bacilli and cocci giving rise to violet, pink, yellow and orange colours.

The number of kinds are found to vary with the season when the cheese is made and the source from which the milk is derived. Of this miscellaneous group, *B. lactis aerogenes* is the most commonly found: typical *B. coli* is rare.

*Fungi*. In the course of our investigations we have observed a

considerable number of species of fungi, those of most frequent occurrence being *Oospora lactis*, *Mucor mucedo*, *Aspergillus glaucus*, *Cladosporium herbarum*, *Penicillium glaucum*, and several forms of *Torula*. The four first mentioned appear only on the coat of the cheese, rarely or never in the interior. They may be regarded as accidental or unavoidable in the ordinary course of manufacture of Stilton cheese, and apparently play little or no part in the ripening process.

*Oospora* is very abundant on the outside during the first 15 to 20 days, when the coat of the cheese is moist.

*Mucor*, *Aspergillus*, and *Cladosporium* are more casual in their occurrence, and may appear upon cheese of all ages in small irregular numbers.

The fungi which are undoubtedly of great importance in the ripening process of Stilton cheeses are *Penicillium glaucum* and one or two forms of *Torula*; these are found throughout the interior of the cheese.

When cut across a well-made ripe Stilton exhibits a characteristic mottling of blue veins most abundant in the softer centre, and radiating in an irregular manner towards the firmer outside. These veins are crevices more or less filled with the mycelium and conidiophores of *Penicillium glaucum*. We have observed conidia in small numbers in the fresh curd; they are, however, very sparsely distributed and difficult to find, and the fungus rarely occurs on plates inoculated from cheeses less than three weeks old. Either from want of air or from the presence of inhibiting substances the spores appear to be prevented from germinating in the closely packed curd in which they are imbedded. In the shrinking cheese cavities arise at the points where the separate pieces of curd first packed in the mould touch each other: it is in these cavities that the mycelium of the fungus makes its appearance and spreads over the surface of the crevices at a rapid rate without penetrating into the substance of the cheese more than a very small fraction of an inch.

The mycelium is colourless, and it is not till the formation of the conidiophores and the conidia that the blue-green tint of the "veins" in the cheese is developed.

In open spaces with plenty of room for growth the conidiophores are of the ordinary type, the hyphae being about  $3\mu$  in diameter, with sterigmata  $5.5-8\mu$  long, bearing round smooth conidia  $2.8-4\mu$  in diameter. When seen in mass the conidia are of a bluish-green tint:

grown on agar or gelatine media the blue-green tint of the colonies ultimately changes to a greyish brown or mouse colour.

We found that in many of the crevices, especially those of small dimensions, the hyphae of the mycelium become much thickened, their diameter reaching  $9-12\ \mu$ . The apex of such thickened hyphae may give rise to a much thinner hypha which develops into a conidiophore (Fig. 7 *b*). Sometimes sterigmata and conidia arise on a very short hypha placed laterally upon the thick mycelial hypha as in Fig. 7 *a*. Not infrequently we have observed the development of a single sterigma with its conidia within the individual cells formed by partitions across the thickened hypha: the protoplasm of the cell shrinks, leaving an enclosed space, into which the fructification develops later (Fig. 7, *c* and *d*), such remarkable phenomena not only occurring at the apex of a hypha but sometimes in cells along the hyphae at random. Ascocarps have not been found.

We find that *Penicillium glaucum* does not grow on agar lactose media when the latter is inoculated at the same time with the Stilton *Tyrothrix*, although it grows luxuriantly enough in the presence of lactic acid organisms. The conidia germinate but the hyphae produced remain very short where *Tyrothrix* is growing freely; later, after the formation of spores by the *Tyrothrix*, the fungus develops rapidly.

We hope to further study the influence of these organisms upon each other.

*Yeasts.* One of the most frequent constituents of the micro-flora of Stilton cheese is a species of *Torula*. It occurs in cheeses of all ages, being abundant in those 24 hours old, as well as in those which are completely ripe.

The fungus is not only found on the coat, but is distributed throughout the interior of the cheese.

Plates inoculated with dilutions prepared from ripe cheeses exhibit colonies of the *Torula* in abundance, along with those of *Penicillium* and a few bacteria.

The cells of the Stilton *Torula* are round,  $3.5-5\ \mu$  in diameter (Fig. 6).

Growth takes place more freely upon acid media than upon alkaline substrata. The colonies are round, white, and opaque, with smooth shining surfaces.

Less frequently met with is another form of yeast-like fungus, with oval cells  $5.5-6\ \mu$  long and  $3.5-4\ \mu$  broad. The colonies are white and opaque, with dull matt surfaces, which ultimately become wrinkled.





Fig. 1.



Fig. 5.

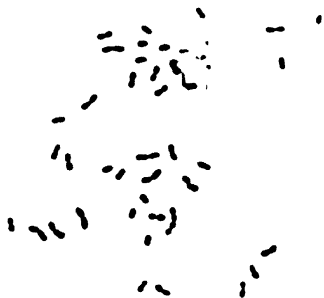


Fig. 2.

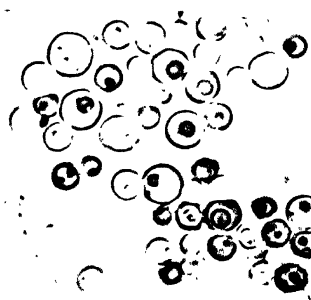


Fig. 6.



Fig. 3.

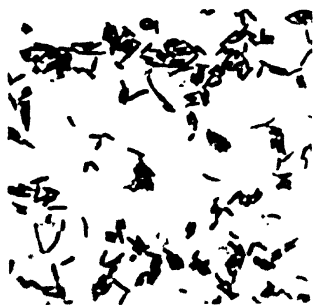


Fig. 4.



Fig. 7.

We have not been able to induce the formation of endospores in either of these "yeasts."

### Summary.

1. The numbers of bacteria and fungi in a newly-made Stilton may rise to the enormous number of 1000 to 3000 millions per gram in the first week.

2. There is a gradual fall in the numbers up to the time of ripeness (100 to 150 days old), when 50 to 100 millions only are found.

3. In the early stages lactic acid bacteria are most abundant. When the cheese is ripe the lactic acid bacteria are few and weakened in physiological power; *Penicillium glaucum* and a form of *Torula* are then abundant.

4. Five characteristic organisms are found in all Stilton cheeses examined, viz.:—(1) *Streptococcus lacticus*, (2) a short rod-shaped form of *Bs. acidi lactici*, (3) a species of *Tyrothrix*, (4) *Penicillium glaucum*, and (5) a round form of *Torula*, sometimes accompanied or replaced by an oval form.

In cheeses where starters have been used we find a large celled form of *Streptococcus lacticus* present.

5. *Penicillium glaucum* is checked in its growth by the *Ty-rothrix*.

We wish to acknowledge our indebtedness in this investigation to a grant made from the Development Fund by the Board of Agriculture and Fisheries.

### EXPLANATION OF FIGURES IN PLATE VI.

The photomicrographs were taken with a 2 mm. Zeiss apochromatic objective and No. 8 eye-piece by Mr F. O. Mosley, University College, Reading.

- Fig. 1. Small common form of *Streptococcus lacticus* from agar,  $\times 900$ .  
 „ 2. Large form of *Streptococcus lacticus* from agar,  $\times 900$ .  
 „ 3. „ „ „ „ milk,  $\times 900$ .  
 „ 4. Short-rod lactic organism from agar (*Bs. acidi lactici* Hueppe),  $\times 900$ .  
 „ 5. Stilton cheese *Tyrothrix* from agar,  $\times 900$ .  
 „ 6. *Torula* from Stilton cheese from agar culture,  $\times 900$ .  
 „ 7. Abnormal forms of hyphæ and conidiophores of *Penicillium glaucum* from interior of Stilton cheese.



## FACTORS AFFECTING SUSCEPTIBILITY TO DISEASE IN PLANTS.

### PART I.

By G. T. SPINKS, B.A.,

*Trinity Hall, Cambridge, Development Grant Research Scholar.*

THE problem of immunity to disease in plants is now receiving a large amount of attention from plant-pathologists. It has long been known that different varieties of the same species of plant may differ in their susceptibility to the attacks of fungi and possibly other parasites, but even now practically nothing is known of the factors which determine whether a plant will prove immune to a disease or not. The following paper is a record of investigations which it was hoped would contribute something towards the solution of this problem, or which might at least suggest lines of further research on this subject.

It was thought that the simplest method of starting an attack on this question was by finding what effect the nutrition of a plant had on its susceptibility to disease. There has long been a general impression that heavy manuring makes plants more liable to disease, but hitherto no definite data have been available nor any detailed experiments made.

From experiments on the effect of mineral starvation on *Bromes* Marshall Ward<sup>1</sup> concluded that decreased susceptibility to rust was only caused by the lack of sufficient food in the host plant on which the rust fungus could feed, i.e. that the host was just as susceptible as under normal conditions but could not supply food to so large a quantity of the rust fungus. Later, histological examinations also showed that fungal hyphae in an immune plant were adversely affected in the same way as in a starved susceptible plant<sup>2</sup>.

<sup>1</sup> M. Ward, *Proc. Roy. Soc.* 71, 1903.

<sup>2</sup> M. Ward, *Ann. Bot.* 19, 1905.



These seemed to be the only records of any investigations on the effect of nutrition on immunity to disease in the higher plants. It was therefore decided to grow numbers of wheat plants under varying conditions of nutrition and to observe any differences in their susceptibility to the attacks of disease.

The first set of experiments was performed with a number of wheat plants grown in water-cultures. The wheat grains were germinated on damp blotting-paper and were transferred, when the plumules were about an inch long, to the water-cultures. The young plants were supported in split corks in the necks of the bottles which had a capacity of about 300 c.c.

Throughout the experiments the bottles were filled up with tap-water on alternate days to replace the water lost by transpiration: the solution was left in the bottles for ten days without any attention except the filling-up above mentioned, and was then poured out and replaced by clean tap-water. After two days the bottles were filled with fresh nutrient solutions. These operations were repeated throughout the experiment, so that the plants received fresh solutions every twelve days but were in tap-water only for the last two days of each twelve-day period. It should be mentioned that the plants grew exceedingly well under these conditions and were indeed remarkable for their strong growth and extensive tillering. All the bottles received the same standard nutrient solution but to different series were added various small amounts of strong solutions of different salts. The standard solution employed was the one used by Detmer and made up as follows:

Calcium nitrate	1 gm.
Potassium chloride	·25 gm.
Magnesium sulphate	·25 gm.
Potassium dihydrogen phosphate	·25 gm.
Ferric chloride	a few drops of solution.
Tap-water	1000 c.c.

Fifteen series of cultures were grown, each series consisting of three plants of a variety of wheat highly susceptible to *Puccinia glumarum* (Michigan Bronze) and three of a variety almost immune to the attacks of this fungus (Little Joss).

Series 1 was supplied with the normal nutrient solution, the 300 c.c. of solution which was placed in each bottle containing ·051 gm. nitrogen in the form of nitrate, ·017 gm. phosphorus as phosphate, and ·06 gm.

potassium. In series 2 the amount of nitrogen was doubled by adding .3 gm. sodium nitrate, containing .051 gm. nitrogen, to the solution in each bottle, while in series 3 the amount of nitrogen was increased to four times the normal by adding .9 gm. sodium nitrate. The next two series, 4 and 5, also contained double and four times the normal amount of nitrogen, but in this case the additional nitrogen was contained in .24 gm. and .72 gm. of ammonium sulphate respectively. To series 6 and 7 was added sodium phosphate, the addition of .077 gm. containing .017 gm. phosphorus doubling the phosphorus in series 6, and of .23 gm. making the phosphate four times the normal in 7. Similarly the potassium in series 8 and 9 was increased to twice and four times the normal by adding .115 gm. and .345 gm. of potassium chloride, that is .06 and .18 gm. potassium, to series 8 and 9 respectively. Series 10 and 11 received only the normal solution but the concentration in 10 was double, and in 11 four times the normal concentration. On the other hand series 12 and 13 received the normal solution diluted to one half and one quarter the original concentration. Series 14 and 15 received an addition of both potassium and phosphate; in the first case the potash and phosphate were both brought to twice the normal, and in the second case to four times.

The seedlings were placed in the water-cultures on Feb. 28 in the case of the series 1—9, and a week later in the case of the remaining series, the whole set of cultures being grown close together in the same greenhouse.

As may be deduced from the varieties of wheat selected, it was originally intended to inoculate the plants with *Puccinia glumarum*, but as late as April 24 it had been found impossible to obtain enough to infect the plants. Only a very small quantity of this fungus was obtained on April 24 and a few inoculations were made, but these proved to be failures. In the meantime a spontaneous outbreak of *Erysiphe graminis* had occurred among the plants, so it was resolved to study the attack of this parasite instead of that of *Puccinia glumarum*. Accordingly the spread of the mildew was encouraged by putting the plants close together and keeping the atmosphere in the greenhouse damp. Also, the relative positions of the plants were frequently changed so as to give the healthy every opportunity of being attacked, and at the same time to eliminate any differences due to the outside plants receiving more air and light.

On April 24 all the plants were examined and marks were awarded to each in proportion to the amount of mildew present, those with only

a trace of mildew being marked 1 and those which were most severely attacked being marked 10. This examination and marking was repeated on May 3 and for the third and last time on May 15. By this time the most badly attacked plants were covered all over with a thick coating of mildew and were practically dead: their condition can be seen from the photograph in Plate VII, Fig. 1. The least diseased plants only showed a few patches of mildew and their leaves were strong and of a good green colour, contrasting very strongly with the dingy flaccid leaves of the badly diseased plants. Fig. 2, Plate VII, shows one of the healthiest plants.

It must be remembered that these comparatively healthy plants had been surrounded by, and actually in contact with, the badly diseased ones for several weeks and had thus had every opportunity of succumbing to the attack of the parasite. Most of the plants grew to about the same size, the exceptions being in the series 5, 11, 12 and 13.

The plants in series 5, which received a large addition of ammonium sulphate, were of the average size as regards their leaves but their root-systems were distinctly poor. The plants growing in the concentrated solution in series 11 were rather smaller and their roots were rather below the average size. The plants which grew in solutions more dilute than the normal (12 and 13) were small but had well-developed roots.

The intensity of disease increased on all the plants between the first and last occasion of marking, but as the relative positions of the plants in the scheme of marking were practically constant only the final markings are given here.

Table I shows the marks which indicate the amount of disease on each of the 90 plants at the conclusion of the experiment.

It will be seen from the preceding table that the nutrient solutions in which the plants were growing exercised a very large influence on their susceptibility to disease, and that the different degrees in which the plants became diseased was not accidental but can be correlated with the treatment they received. In the first place it will be noted that although one variety of wheat was more susceptible to disease than the other, yet the effect due to the different solutions follows almost the same relative order in both varieties. In both cases series 2, 3, 4 and 5 which received additional nitrogen contained the most badly diseased plants (Plate VII, Fig. 3), though it is curious that the plants of No. 5 receiving four times the normal amount of nitrogen as ammonium sulphate were not so badly attacked as those of No. 4 which only



Fig. 1.



Fig. 2.



Fig. 3.

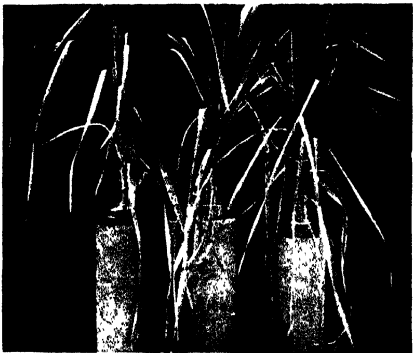


Fig. 4.



Fig. 5.



Fig. 6.



received twice the normal amount of nitrogen: perhaps there is some connection between this fact and the stunted root-growth noticed in series 5.

TABLE I.

No.	Solution	Degree of disease					
		Little Joss (3 plants)			Michigan Bronze (3 plants)		
1	Normal . . . . .	9	7	7	10	5	5
2	Nitrogen $\times 2$ } $\text{NaNO}_3$ . . . .	9	9	9	10	7	7
3	" $\times 4$ }	10	10	8	10	7	5
4	" $\times 2$ } $(\text{NH}_4)_2\text{SO}_4$ . . . .	10	10	10	9	9	9
5	" $\times 4$ }	8	8	8	8	8	5
6	Phosphate $\times 2$ . . . . .	6	6	4	6	5	5
7	" $\times 4$ . . . . .	7	7	7	5	5	2
8	Potash $\times 2$ . . . . .	7	5	4	5	5	2
9	" $\times 4$ . . . . .	5	5	3	3	3	3
10	Normal $\times 2$ . . . . .	9	9	9	9	7	7
11	" $\times 4$ . . . . .	9	8	8	5	5	5
12	" $\times \frac{1}{2}$ . . . . .	7	7	5	1	1	1
13	" $\times \frac{1}{4}$ . . . . .	7	5	5	1	1	1
14	Phosphate and potash $\times 2$ . . . . .	7	7	7	5	3	3
15	"   "   " $\times \frac{1}{2}$ . . . . .	7	4	4	7	5	5

Next, the plants receiving a concentrated nutrient solution were observed to be badly diseased, but here again the extremely strong solution in series 11 has checked the growth of the plants to some extent, and at the same time there is less disease than in the plants of No. 10 (Plate VII, Fig. 4).

Series 6 and 7 receiving additional phosphate showed about the average amount of disease, or possibly rather less. The series 8 and 9 which received additional potash were very clearly rendered less susceptible to disease: in the case of Little Joss the plants of Nos. 8 and 9 were the healthiest of the whole set (Plate VII, Fig. 5), while among the Michigan Bronzes they were the healthiest with the exception of those of series 12 and 13.

The plants receiving additional phosphate and potash (14 and 15) seemed to be intermediate in their susceptibility between those receiving only phosphate or only potash.

The plants growing in dilute solutions (12 and 13) were healthier than the normal, and indeed in the case of the Michigan Bronzes they were the healthiest of the set (Plate VII, Fig. 6): in both varieties the plants of series 13 were slightly less diseased than those of 12; that is, the disease was reduced when the food supply was reduced.

While the above-mentioned water-cultures were in progress a parallel series of experiments was being made on wheats grown in soil.

A number of wooden boxes about 17 inches  $\times$  13 inches were filled to a depth of  $2\frac{1}{2}$  inches with rather poor soil which was obtained by mixing a rather rich soil, obtained from the neighbourhood of the laboratory, with about an equal bulk of sand. In one box the soil was made still poorer by doubling the amount of sand.

On March 12 twelve grains of Little Joss were sown in each box and twelve of Michigan Bronze, two rows of each variety in each box : on the average eleven grains of each variety produced plants. The boxes were all in the same greenhouse near the water-cultures, so that the plants were very readily naturally infected by the mildew, and in this case also the position of the boxes was often changed in order to make the conditions as far as possible equal for all.

An attempt was made to add artificial manures to the boxes in amounts comparable to those used in practice in the field. It was calculated that the area of a box was  $1\frac{1}{2}$  square feet or  $\frac{1}{30,000}$  acre and that 1.7 grams per box would be equivalent to 1 cwt. per acre. The quantities of fertilisers applied are given in Table II which also records the results. The salts to be added were made into solutions and it was intended to apply these solutions in six doses at intervals of a fortnight, but the experiment was brought to an end when only four of these doses had been applied. This explains the fractions in the amounts of fertiliser applied ; thus 1.33 cwt. was originally intended to have been increased to 2 cwt., and so on.

The plants in all the boxes grew almost equally well, but before the mildew had attacked them seriously those which received nitrogenous manures were larger and of a darker colour than the rest : on the other hand the plants in the "starvation" box were small, thin and light-coloured. As in the case of the water-cultures the boxes were examined and graded according to the amount of mildew present on May 4, 15 and 20, but as the relative amounts were constant only the last set of marks is given here. The plants in each box were not marked individually, as the individuals of the same variety in the same box showed no differences, but the two varieties in each box were judged separately.

The extent of the disease on the plants in each box is shown in Table II.

It will be seen that the order of the severity of the attack of mildew in the different boxes was the same for both varieties of wheat.

The plants in box 5 receiving a double dressing of sodium nitrate were the most severely attacked in both cases, while all the other boxes which received nitrogenous manures showed a very bad attack. The least susceptible plants were those in box 9, which received a double dose of sodium phosphate, while those in 10 and 11 which received potassium chloride were only slightly attacked, the same being the case in box 14 where the plants were almost starved.

TABLE II.

No.	Treatment	Degree of disease	
		Little Joss	Michigan Bronze
1	No manure . . . . .	7	5
2	Complete manure 2.3 gm. $\text{NaNO}_3$ equivalent to 1.3 cwt. $\text{NaNO}_3$ per acre 3.4 „ $\text{Na}_2\text{HPO}_4$ „ „ 3.3 „ „super” „ 27 „ KCl „ „ 1.3 „ kainit „	9	7
3	Complete manure. Double the above quantities .	9	7
4	2.3 gm. $\text{NaNO}_3$ equivalent to 1.3 cwt. $\text{NaNO}_3$ per acre ..	8	6
5	4.6 gm. $\text{NaNO}_3$ equivalent to 2.6 cwt. $\text{NaNO}_3$ per acre .	10	8
6	18 gm. $(\text{NH}_4)_2\text{SO}_4$ equivalent to 1 cwt. $(\text{NH}_4)_2\text{SO}_4$ per acre	8	6
7	3.6 gm. $(\text{NH}_4)_2\text{SO}_4$ equivalent to 2 cwt. $(\text{NH}_4)_2\text{SO}_4$ per acre	9	7
8	3.4 gm. $\text{Na}_2\text{HPO}_4$ equivalent to 3.3 cwt. „super” per acre	6	4
9	6.8 gm. $\text{Na}_2\text{HPO}_4$ equivalent to 6.6 cwt. „super” per acre	3	1
10	27 gm. KCl equivalent to 1.3 cwt. kainit per acre . . . . .	4	2
11	54 gm. KCl equivalent to 2.6 cwt. kainit per acre . . . . .	5	3
12	3.4 gm. $\text{Na}_2\text{HPO}_4$ equivalent to 3.3 cwt. „super” per acre 27 „ KCl „ „ 1.3 „ kainit „	7	5
13	Double the quantities in No. 12 . . . . .	6	4
14	No manure. Double the quantity of sand . . . . .	5	3

A single dressing of sodium phosphate seems to be less useful in preventing disease than a single dressing of potassium chloride, but if the dose be doubled the result is reversed.

A mixture of sodium phosphate and potassium chloride also seems to be rather less effective than either of these substances separately.



On the whole these results agree very closely with those obtained from the water-cultures, though phosphates appear to be more effective in checking disease when the plants are grown in soil than when grown in water-cultures.

Two more sets of wheat-cultures were carried out in a similar manner to the preceding ones in order to find the effect of nutrition on the susceptibility of the plants to yellow rust, *Puccinia glumarum*.

The first of these was a set of water-cultures in which the same varieties of wheat as before were used, but a much smaller number were employed. In this case only those culture solutions were used which had been found to give extreme results in the first investigation, together with one of the so-called normal solutions as a control. There were then three series of cultures, each consisting of three plants of each variety, and they were treated in identically the same way as the corresponding cultures of the former set. The only difference was that this set of cultures was carried out in the open air as far from the greenhouse as possible in order to avoid infection by the mildew: but it was found impossible to keep them free from the mildew, though they were not nearly so badly attacked as those on which this parasite had been encouraged.

The plants of series 1 received the normal solution (cf. series 1 of former set); those of series 2 had the amount of nitrogen in the solution doubled by the addition of ammonium sulphate (cf. series 4 above), and those of series 3 were grown in a solution containing four times the normal amount of potash (cf. series 9).

On May 23 the seedling wheats were placed in the water-cultures and on May 28 when each showed two leaves they were inoculated with uredospores of *Puccinia glumarum*. The inoculations were made by scraping a "rusty" leaf of wheat with a wet penknife-blade and then applying the blade to the tips of the leaves to be infected; the leaf-tips were first made moist by blowing on to them through a narrow glass tube which caused the water containing the spores to adhere more readily to the leaf. On June 6 more inoculations were made, but this time on the middle of the upper surface of the leaf-blades: there were at this time three or four leaves on each plant.

On June 10 rust pustules appeared on one plant on an inoculated spot, and on June 12 eight plants showed pustules. From this date the disease spread rapidly without any artificial assistance other than that of placing the plants very close together so that the rusty leaves could touch the healthy ones.

The plants were graded for rust on July 1 and it is interesting to note that at this time all the plants showed rust pustules, though the Little Joss, which is almost immune, had the disease to a very much less extent than the Michigan Bronze. On July 12 the plants were again marked for the rust attack and the marks are shown in Table III.

TABLE III.

No.	Solution	Degree of rust					
		Little Joss			Michigan Bronze		
1	Normal .....	0	0	0	8	6	5
2	Nitrogen 2 (Ammon. sulph.)	0	3	3	9	9	8
3	Potash 4 ..	0	0	2	6	5	3

It will be seen that only three out of the nine plants of the relatively immune variety showed any rust at this stage, and of these two were those which had received solutions with double nitrogen. With one exception all the Michigan Bronze plants were much more diseased than any of the other variety, and it will be seen that the attack on series 2 was much worse than that on 1, while on series 3 it was slightly less. By this time all the plants of the first variety were suffering severely from mildew, so they were thrown away: but the plants of the other variety were kept for a few weeks longer. On July 23 the remaining plants were again marked and found to occupy the same relative positions as in the previous marking. On Aug. 5 however the rust on the plants of series 1 and 2 was the same as on the previous occasion, while two of the plants in series 3 were much more severely attacked than before, earning marks 8 and 6. Perhaps this can be accounted for by the fact that the plants of 1 and 2 were almost killed by mildew and so the rust had no chance to spread further, while plants of series 3 were still green and growing vigorously.

Observations were also made on the susceptibility to rust of a number of wheat plants grown in pots receiving different nutritive solutions. Fourteen series of 5-inch pots were grown, each series consisting of one pot containing three plants of Little Joss and one containing three plants of Michigan Bronze. The amounts of manurial substances to be added to the various pots were calculated on the same

system as was employed in the case of the boxes. As before, a soil mixed with about an equal amount of sand was used in all the pots.

The fourteen series of pots were treated in the following ways: No. 1 was a control receiving no manure; Nos. 2, 3 and 4 received different quantities of a complete manure; Nos. 5 and 6 received respectively a single and double dose of sodium nitrate, while 7 and 8 were given the same amounts of nitrogen in the form of ammonium sulphate. To Nos. 9 and 10 were given single and double dressings of sodium phosphate, and Nos. 11 and 12 received similar treatment with potassium chloride. Nos. 13 and 14 received no manure, but were sterilised in a steamer at 100° C. for two hours before the seeds were set. The manures used were equivalent to about 2 and 4 cwt. sodium nitrate per acre, 1½ and 3 cwt. ammonium sulphate, 5 and 10 cwt. superphosphate, and 2 and 4 cwt. kainit. The fertilisers in solution were added in three separate doses, an interval of about a fortnight occurring between the applications, and the first dressing was given about a week after sowing the seeds.

The seeds were sown in the pots on May 8 and by May 20 all the seeds had germinated and each seedling showed one or two leaves. Between May 18 and 23 every leaf on all the plants was inoculated with uredospores of *Puccinia glumarum* in the manner previously described. The first sign of successful infection was seen on May 28, when one leaf was found to bear some unbroken pustules on its tip: these pustules opened and set free their spores on the following day. More leaves gradually showed pustules until on June 5 the disease was evident on Little Joss plants in four pots, and on Michigan Bronze plants in eight pots. On June 6 another inoculation was made, this time on the middle of the upper surface of the leaves, about four leaves being inoculated in each pot.

By June 10 all the Michigan Bronze plants showed rust, while the disease had appeared on about half the Little Joss plants. From this date onward the disease spread rapidly and on July 1 the cultures were first graded according to the amount of rust present on each. At this time ten of the fourteen Little Joss cultures were marked 0 as being free from rust, while the remaining four were marked 1 or 2, the disease attack being very slight. Among the Michigan Bronze cultures 9, 10, 11 and 12 were the least diseased, all the others being almost equally bad, although perhaps 3, 7 and 8 were the worst.

On July 10 the marks awarded were almost the same as before, but

although there were again four plants of Little Joss showing degrees 1 or 2 of rust three of these were not included in the four on July 1.

The plants were again marked according to their rustiness on July 23, Aug. 5 and 13, but the disease was making no further progress and the relative amounts of rust on the various plants showed only small fluctuations which might almost be put down to errors in observation. The figures in Table IV may therefore be considered to represent the extent of the disease on the different cultures when the disease had attained its maximum development.

TABLE IV.

No.	Manure equivalent per acre	Extent of disease	
		Little Joss	Michigan Bronze
1	None .. ..	0	6
2	1 cwt. sodium nitrate } 2½ „ „super” } 1 „ „kainit }	1	7
3	2 „ sodium nitrate } 5 „ „super” } 2 „ „kainit }	1	7
4	4 „ sodium nitrate } 10 „ „super” } 4 „ „kainit }	1	7
5	2 „ sodium nitrate	1	7
6	4 „ „ „	1	7
7	1½ „ ammonium sulphate	1	7
8	3 „ „ „	1	9
9	5 „ „super”	1	5
10	10 „ „ „	1	6
11	2 „ „kainit	1	5
12	4 „ „	1	4
13	None. Soil sterilised	1	7
14	„ „ „	1	7

At first it was thought that only two or three of the Little Joss cultures showed any sign of disease, but a very careful scrutiny revealed

minute traces, perhaps three or four unbroken pustules, on each culture except No. 1. It was noted that these pustules always appeared on leaves which were almost dead, and which therefore apparently had not quite the same power of resisting the disease that a leaf in full vigour possesses. This wheat then was so nearly immune to the disease under all the conditions of the experiment that no difference was perceptible between the various cultures.

The differences between the susceptibility of the various cultures of Michigan Bronze were not nearly so striking as they were in the experiment where mildew was used, but the marks in Table IV show that some differences existed. Nos. 11 and 12, receiving potash, were clearly the least diseased, though 9 and 10 which were treated with sodium phosphate were nearly as free from disease. All the cultures which received nitrogenous dressings were about equally diseased, except No. 8 which was considerably worse than any other. The cultures in the sterilised soil showed more disease than the control plants and were about equal to most of those to which nitrogen was given.

It was thought desirable to compare the preceding results with those obtained on a larger scale, and we were enabled to examine the field-plots on the farm of the Royal Agricultural Society at Woburn for this purpose. The pot-cultures at Woburn were also examined and gave some interesting results, though the treatments they had been receiving were quite different from those applied to any of our own cultures.

With regard to the pot-cultures, it was found that rust was practically absent, the amount present being so small that no idea of the susceptibility of the various cultures could be gained.

Table V shows the marks denoting the amount of mildew on the various wheat cultures and their mode of treatment.

It is seen from the above table that the severity of the attack on the control plants varied between 2 and 5 with an average of 3 to 4. Only one of the cultures receiving basic slag was marked outside this range, while the average marks were about the same: the addition of basic slag seems therefore to have had no effect on the susceptibility of the plants to mildew. The plants grown in the soil containing magnesia showed a distinct increase of susceptibility to the disease (8) which was very little, if at all, decreased by the addition of lime. These plants were much greener and less mature than those of most of the cultures, which were beginning to ripen. It is possible that an increased

TABLE V.

Cultures	Treatment	Mildew
38 and 41	Control .. .. .	4, 5
42-47	Basic slag added in various ways .	5, 1, 1, 3, 1
56-63 and 68	" " " " ..	4, 4, 2, 4, 3, 3, 3, 4, 2
80-86, 96-100	" " " " ..	3, 1, 3, 4, 4, 3, 5, 4, 2, 2, 4, 4
50	Soil containing MgO .	8
51-55	" " " (CaO added)	8, 7, 7, 7, 7
101 and 102	Control .. .. .	3, 3
102-108	'03 - '001 % Lithium phosphate	0, 0, 0, 0, 1, 1
109-114	'03 - '01 % Zinc phosphate . . . .	4, 4, 5, 4, 5, 4
115, 117, 119	'03 - '01 % Lead " ..	3, 3, 3
121	Control .. .. .	4
123, 125, 127	'03 - '001 % Lithium nitrate . . . .	0, 0, 6
129-134	'03 - '01 % Zinc nitrate .	9, 10, 10, 10, 10, 10
135-140	'03 - '01 % Lead " ..	7, 7, 7, 5, 5, 5
141 and 142	Control .. .. .	2, 2
143-148	'03 - '001 % Lithium carbonate	0, 0, 1, 0, 1, 1
149-154	'03 - '01 % Zinc carbonate	3, 3, 3, 1, 3, 2
155-160	'03 - '01 % Lead " ..	3, 1, 3, 1, 1, 1

The cultures treated with zinc salts showed interesting differences: those receiving zinc carbonate were slightly below the normal in the amount of disease, the phosphate increased the susceptibility slightly, while on those plants treated with zinc nitrate the disease was extremely bad and had almost killed the plants. The amount of zinc salt applied did not affect the amount of disease present. The plants treated with zinc nitrate were much shorter and less mature than other plants, and were said to have been very severely checked in the early stages of their growth; so it is possible that this, rather than any direct action of the zinc nitrate, may have been the cause of the increased disease attack.

The lead salts also varied in their effects on the disease. The plants receiving the phosphate scarcely differed from the normal; the nitrate increased the disease distinctly but not as much as the zinc nitrate did; and the carbonate decreased the attack slightly. The amount of lead nitrate added produced corresponding variations in the amount of disease present. The carbonates of all the above metals seemed to diminish the susceptibility of the wheat slightly.

The field plots of wheat were unfortunately not examined until late in the season when the wheat was almost ripe; therefore in many cases it was extremely difficult to determine how much disease there had been, especially as regards mildew. Table VI shows the treatment the various plots had received and the amount of mildew and rust on them: where no figure is given for the mildew the wheat was so ripe that no trustworthy figures could be obtained.

TABLE VI.

Plot	Manures applied annually per acre	Mildew	Rust
1	Unmanured	1	1
2 a	Sulphate of ammonia (= 25 lbs. $\text{NH}_3$ )		
2 aa	As 2 a with 5 cwt. lime 1905, 1909, 1910, 1911	1	4
2 b	" " 2 tons lime 1897	1	4
2 bb	" " " 1897, 1905	6	5
3 a	Nitrate of soda (= 50 lbs. $\text{NH}_3$ )	8	5
3 b	" " (= 25 lbs. $\text{NH}_3$ )	10	6
4	Minerals (3 cwt. "super" and $\frac{1}{2}$ cwt. $\text{K}_2\text{SO}_4$ )	0	0
5 a	" " and ammonium sulphate (= 25 lbs. $\text{NH}_3$ )	2	4
5 b	As 5 a with 1 ton lime 1905	3	4
6	Minerals and sodium nitrate (= 25 lbs. $\text{NH}_3$ )		3
7	Unmanured	1	1
8 a	Minerals and in alternate years ammonium sulphate (= 50 lbs. $\text{NH}_3$ ) (1911)		6
8 aa	As 8 a with 10 cwt. lime 1905		7
8 b	Minerals and in alternate years ammonium sulphate (1912)		6
8 bb	As 8 b with 10 cwt. lime 1905		8
9 a	Minerals and in alternate years sodium nitrate (= 50 lbs. $\text{NH}_3$ ) (1911)		1
9 b	Minerals and in alternate years sodium nitrate (1912)		4
10 a	"Super" 3 cwt. and sodium nitrate (= 25 lbs. $\text{NH}_3$ )		2
10 b	Rape dust (= 25 lbs. $\text{NH}_3$ )		4
11 a	$\text{K}_2\text{SO}_4$ 1 cwt. and sodium nitrate (= 25 lbs. $\text{NH}_3$ )		2
11 b	Farmyard manure (= 100 lbs. $\text{NH}_3$ )		4

It is perhaps unnecessary to explain that these plots have been growing wheat and have received the same treatment continuously for over thirty years. The wheat grown this year was Square Head's Master.

From the figures available for the mildew it is seen that the unmanured plots 1 and 7, and plot 4 which receives mineral manures only, were practically free from mildew. The crops on these plots were, of course, very thin and short. The ammonium sulphate plots 2aa, 2b and 2bb showed a medium amount of mildew, but the plots on which the disease was worst were Nos. 3a and 3b which receive only nitrate of soda. The plants here were of only medium size, not at all luxuriant in their foliage.

The figures for the mildew cannot be considered to be at all accurate, but the two extremes were clearly enough marked, 1, 7 and 4 on the one hand and 3a and 3b on the other.

The marks for the rust are more to be depended on, and on examining them it is first noticed that the same plots 1, 7 and 4 were again almost disease-free. From the rest of the figures very little is to be gathered: on plots 8a to 8bb the addition of minerals to the ammonium sulphate seems to have increased the tendency to disease as compared with plots 2aa to 2bb which received ammonium sulphate without mineral manures: on the other hand no increased disease was noted on plots 5a and 5b. Addition of minerals to sodium nitrate on the contrary seems to have decreased the amount of disease, as may be seen by comparing plots 6, 9a, 9b, 10a and 11a with plots 3a and 3b. The dunged plot showed about an average amount of rust.

The field plots of barley were much less advanced than the wheat and showed the different degrees to which they had been attacked by mildew very plainly. On two or three of the plots a small amount of *Puccinia graminis* was found, but it was not at all common so no marks were given for the rust attack. The barley plots have received almost exactly parallel treatment to that of the wheat plots except that plots 2b, 5b, 8aa and 8bb were given another dressing of lime this year: also 5a is divided into 5a and 5aa, the latter having received a dressing of lime in 1905. The variety of barley grown this year was Goldthorpe. The marks assigned for the amount of mildew on the barley plots are given in Table VII.

Plots 1 and 4, as we should have expected from our previous experience, were two of the least diseased; but it is at first surprising to find that No. 9a, which received a large dressing of nitrate of soda the previous year in addition to minerals, showed so little disease. In all probability the soluble nitrates had been completely washed out of the porous Woburn soil. The small amount of mildew on the dunged plot is also rather strange, especially as the plants were growing



luxuriantly. The disease was worst on plots 3*a* and 3*b* which received sodium nitrate only, and then on the 8's, which were manured with minerals and ammonium sulphate. The 8's are worse than the 2's which received ammonium sulphate only; but the 5's which also received ammonium sulphate and minerals were only slightly worse than the average. Plots 6 and 9*b*, which were both dressed with sodium nitrate and minerals, showed about the average amount of disease. Where plots received a nitrogenous dressing only in alternate years it was seen that the mildew was worse on the plots which received a dressing this year than on those which were manured in the previous year: cf. 9*a* and 9*b*; 8*a* and 8*b*.

TABLE VII.

No.	Manures applied annually per acre	Extent of mildew
1	Unmanured	3
2 <i>a</i>	Sulphate of ammonia (= 25 lbs. $\text{NH}_3$ )	4
2 <i>aa</i>	As 2 <i>a</i> , with 5 cwt. lime, 1905, 1909, 1910, 1912	6
2 <i>b</i>	" " 2 tons lime, 1897, 1912	8
2 <i>bb</i>	" " " " 1897, 1905, 1912	6
3 <i>a</i>	Nitrate of soda (= 50 lbs. $\text{NH}_3$ )	10
3 <i>b</i>	" " (= 25 lbs. $\text{NH}_3$ )	8
4	Mineral manures (3 cwt. "super" and $\frac{1}{2}$ cwt. $\text{K}_2\text{SO}_4$ )	3
5 <i>a</i>	" " and sulphate of ammonia (= 25 lbs. $\text{NH}_3$ )	+
5 <i>aa</i>	As 5 <i>a</i> , with 1 ton lime 1905	6
5 <i>b</i>	" " 2 tons lime 1897, 1912	7
6	Mineral manures and nitrate of soda (= 25 lbs. $\text{NH}_3$ )	5
7	Unmanured	5
8 <i>a</i>	Mineral manures, and in alternate years, sulphate of ammonia (= 50 lbs. $\text{NH}_3$ ) (1911)	4
8 <i>aa</i>	As 8 <i>a</i> , with 2 tons lime 1897, 1912	8
8 <i>b</i>	Mineral manures, and in alternate years, sulphate of ammonia (= 50 lbs. $\text{NH}_3$ ) (1912)	4
8 <i>bb</i>	As 8 <i>b</i> , with 2 tons lime 1897, 1912	9
9 <i>a</i>	Mineral manures, and in alternate years, nitrate of soda (= 50 lbs. $\text{NH}_3$ ) (1911)	3
9 <i>b</i>	Mineral manures, and in alternate years, nitrate of soda (= 50 lbs. $\text{NH}_3$ ) (1912)	6
10 <i>a</i>	"Super" 3 cwt. and nitrate of soda (= 25 lbs. $\text{NH}_3$ )	5
10 <i>b</i>	Rape dust (= 25 lbs. $\text{NH}_3$ )	5
11 <i>a</i>	$\text{K}_2\text{SO}_4$ 1 cwt. and nitrate of soda (= 25 lbs. $\text{NH}_3$ )	5
11 <i>b</i>	Farmyard manure (= 100 lbs. $\text{NH}_3$ )	4

\* No crop.

| Observations accidentally omitted.

On the whole perhaps the field plots do not show such decisive results as was expected from a consideration of the water- and pot-cultures. But it must be remembered that in dealing with such plots which have received the same treatment for many years other factors come into play besides the direct effect of the manure on the plant.

Some of the irregularities in the results may be brought about by the different chemical and physical conditions existing in the soils of the various plots as a result of the continuous abnormal treatments they have received. Thus the immediate effect of the manure on the plant is possibly counterbalanced by some of these other factors which cannot be estimated.

The conclusions which can at present be drawn from these investigations may be shortly summarised as follows :

Susceptibility to mildew and yellow rust in wheat, and to mildew in barley, is increased by providing the plants with large amounts of available nitrogen: ammonium sulphate and sodium nitrate seem to be equally effective in this direction.

Mineral manures, especially potash salts, on the contrary decrease the susceptibility to disease but cannot counteract the effect of large quantities of nitrogenous manures.

Plants which are semi-starved as regards nitrogen exhibit a considerable degree of immunity, even if the phosphates and potash are also present only in small quantities.

Lithium salts are also effective in producing immunity, while nitrates of lead and zinc, particularly the latter, render plants extremely susceptible. Other salts of lead and zinc have very little effect on the susceptibility of plants.

A variety of wheat which is almost immune to a disease (such as Little Joss to yellow rust) tends to retain its immunity even when supplied with excess of nitrogenous food-material.

Increased immunity does not appear to be due to a lack of food-material available for the fungus in the host, as suggested by M. Ward, because the plants rendered relatively immune by adding phosphates or potash to their food-supply were as healthy and well-grown as those receiving no such additions.

It yet remains to be seen what physiological explanation can be found to account for the changes in susceptibility which can be produced in plants by the above means.

# ON THE GROWTH OF PLANTS IN PARTIALLY STERILISED SOILS.

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DURING the past few years we have grown a large number of plants in partially sterilised soils alongside of others in untreated soils, in order to compare the total weights of dry matter produced. While the experiments were in progress certain qualitative differences in growth and habits of the plants revealed themselves; we propose in this paper to record these differences and to set out certain of the hypotheses that have been put forward to account for them. It was soon found that several distinct problems in plant physiology were concerned, and that any attempt to test the hypotheses experimentally would involve us in a number of side issues and lead us away from our main purpose. We therefore for the present confine ourselves to a statement of the facts observed, leaving their more complete elucidation for later work.

Partial sterilisation is effected in our laboratory in three ways: by treatment with volatile or easily decomposable antiseptics which are subsequently removed, by heating to a temperature just sufficient to put out of action the factor detrimental to bacteria (about 55° C.), and by heating to a higher temperature (100° C.)<sup>1</sup>.

When seeds are placed in soils so treated it is at once observed that their rate of germination has been affected. Germination is sometimes more and sometimes less rapid than in untreated soils under similar conditions of temperature and moisture, but the exact amount of the acceleration or retardation varies very much with the

<sup>1</sup> The temperatures throughout are ° C.

soil, the seed, the amount of water, etc. Retardation is almost always produced in soils heated to 100° or treated with toluene, but acceleration is often obtained in soils heated to 55° C. Some seeds, however, such as the tomato, are almost always retarded in germination. The effect is generally more pronounced in a moist than in a dryer soil, and more in a rich than in a poor soil. Accelerated germination when it occurs is usually shown only by a certain number of the seeds and not by all, whereas retarded germination may in some cases be shown by all the seeds. Generally speaking the most marked effects are obtained in soils heated to 100°, the next in soils treated with toluene, and the least in soils heated to 55°, but there is a distinct qualitative difference between soils treated with toluene, and those heated to 55°, in that accelerated germination occurs more frequently in the latter, as already stated. These effects are produced in all the soils examined though with marked differences in degree. They are seen only to a comparatively small extent in ordinary arable soils and may indeed require careful experiments to detect them, but they are very obvious in heavily manured or pasture soils.

Turning now to the seedling stage: the differences in the plant depend to a large extent on the soil. In relatively poor soils the plants on the partially sterilised soils are sometimes indistinguishable from those on the untreated soil and sometimes larger in size but otherwise similar. In rich soils marked differences occur, which vary with the method of sterilisation but are most striking in soils heated to 100° C. Seedlings growing on the heated soils have, in comparison with those growing on untreated soils, smaller roots and smaller cotyledons of a darker green colour frequently showing some purple. The cotyledons of tomatoes find a difficulty in shedding their seed cases and spreading out, and they also tend to curl under instead of lying flat (Fig. 2).

When the second leaves appear a new set of differences come in which we have noticed particularly in the case of tomatoes grown in pots.

The young tomato plant often shows a considerable amount of purple pigment, especially on the lower part of the stem and the under surface of the leaves. When this appears growth becomes very slow, so that the plant is considerably stunted in comparison with others free from the pigment. The phenomenon is well known to commercial growers who speak of it as a "hard growth" in contradistinction to the soft, sappy growth of a green plant that has suffered no check or

stunting during its life time. It appears that the purple colour is confined to the epidermis, for on stripping this off the normal green appears.

Some of the conditions necessary for the formation of the purple pigment are associated with the soil, and we find it more frequently in steamed than in untreated soils. Thus, when plants on the untreated soil are of a normal green colour, those on the soil heated to 100° may have purple stems and leaves of a dirty green or very dark colour on the upper surface, and deep purple on the veins and the under surface, this being particularly true of the young leaves. So long as the purple persists, growth is considerably retarded and in consequence the plants on the heated soil grow much more slowly than those on the untreated soil.

These effects are very marked in the dull days of winter. A little later on a striking change sets in. The purple colour rapidly disappears, and forthwith the plant begins to make most remarkable growth. Its leaves still remain darker in colour than those of the plants in the untreated soil, but the colour is now a true green untinged with purple. Every part of the plant grows rapidly (Fig. 3). An astonishing development of fibrous root takes place, far exceeding anything produced on untreated soils: the actual weight of root, however, is not necessarily large (Table VII). The stem becomes very stout and the leaves are large and of great substance. Frequently the nodes shorten so that the plant becomes denser and more compact; this, however, depends somewhat on the plant, occurring invariably with cereals<sup>1</sup> and chrysanthemums, but not with tomatoes. Before long the plant is far heavier than those on the untreated soil. As the plants mature the leaves keep green longer and do not so soon take on their red or yellow colours; the bottom leaves also stay on longer than is the case with plants on untreated soils.

But this vigorous vegetative growth does not cause the fruit to suffer. Our tomato plants in heated soil not only flowered earlier, but produced more flowers, more fruit, and earlier and sweeter fruit; they also lasted longer and fruited longer than those in the untreated soil. Earlier flowers and fruit were also obtained from cucumbers, and earlier and larger flowers of lighter colour from chrysanthemums, when these plants were grown in partially sterilised soils.

It commonly happens in late spring and summer, when the light is good and conditions are favourable to plant growth, that the

<sup>1</sup> Measurements for rye are given in this *Journal*, 1909, **3**, 122.

retardation does not appear at all, but the plants go ahead right from the start. We have not yet discovered the precise conditions under which this takes place, and we can never predict with certainty whether retardation or acceleration of growth will set in during the first few weeks in heated soil. Apart from its physiological interest the retardation is of great importance in the practical applications of partial sterilisation because of its frequent occurrence in the early season when the grower under glass is trying to push his plants ahead as quickly as possible. We cannot too strongly emphasise the fact that these remarkable and apparently interchangeable accelerations and retardations *are only met with in the early stages of plant growth*. Later on the rate of growth is governed by the rate of production of ammonia and nitrate in the soil.

If the plants are cut and analysed at any time during the period of active growth those on the heated soil are found to contain a higher percentage of nitrogen and sometimes of phosphoric acid, and the difference extends also to the fruit. When growth is ended and the plant is dead the translocation of nitrogen, phosphorus and potassium from the root and stem to the fruit is commonly found to have been more complete on the heated than on the untreated soil.

With some modifications in detail the same kind of results are obtained with all other plants that we have tried. Mustard is much retarded in growth during its early days in heated rich soil, and takes on a very dark green unhealthy colour. We have not observed the purple patches so prominent in tomato plants, but instead the leaves develop a remarkable tendency to curl towards the under surface; so long as this happens growth is retarded. But later on the leaves open out and vigorous growth takes place, so that the plants soon surpass those on the untreated soil, and, as in the case of tomatoes, they contain a higher percentage of nitrogen, and sometimes of phosphoric acid, in their dry matter. Grasses take on a deep green colour and are commonly retarded in growth but their leaves did not in our experiments show the peculiar curling.

Chrysanthemum cuttings do not generally "strike" as readily in heated as in untreated soil, there being a marked delay, as before, in the formation of root. But once root development begins it continues rapidly till after a time the usual fibrous mass of root is formed (Fig. 4).

Soil heated to 55° behaves entirely differently as a medium for plant growth. The retarded stage is either not induced or is of very brief

duration and the plants rapidly surpass those on the untreated soil and on the soil heated to 100° (Fig. 5). Sometimes growth is particularly rapid giving rise to plants which are extraordinarily large considering their early stage (Fig. 6). There is no characteristic appearance associated with the plants and, except for their larger size, their rather earlier flowering, and more prolific fruiting, they are indistinguishable from those on the untreated soil, and show none of the peculiarities of the plants on the soil heated to 100°. They do not make the sudden late growth seen on soils heated to 100°, nor do they even maintain the rapid growth of their early days, and in the end they are inferior in weight to plants grown on steamed soils. The amount of growth, as before, is conditioned by the amount of ammonia and nitrate made.

Soils treated with toluene sometimes behave qualitatively very much like soils heated to 55°, but on rich soils retardation may be induced in the early stages. In such cases the cotyledons are smaller than usual, dark green in colour and the edges show a strong tendency to curl towards the under surface. The final growth may be greater or less than on soils heated to 55°, but is always less than on soils heated to 100°.

Other volatile antiseptics behave like toluene.

In all these cases the plants contain a higher percentage of nitrogen and sometimes of phosphoric acid in their dry matter than those on untreated soils; they also generally show more complete translocation to the fruit.

Both phenomena are less marked than in soils heated to 100°.

A careful study of the preceding observations brings out seven important directions in which partially sterilised soils differ from untreated soils:

1. There is generally a retardation in germination but sometimes partial acceleration (*i.e.* affecting some of the seeds only).
2. There is generally an acceleration in growth up to the time of the appearance of the 3rd or 4th leaves, but sometimes a marked retardation, especially in rich soils heated to 100° C. We have failed to discover the conditions regulating the retardation and can never predict with certainty whether or not it will set in. On the whole we have observed it more frequently during dull winter days than in the brighter spring or summer days.

3. When this retardation occurs it is accompanied by a very dark green leaf colour and either the formation of a purple pigment or a tendency for the leaves to curl towards the under side. The whole appearance is strongly suggestive of an attempt on the part of the plant to reduce assimilation.

4. Later on the purple colour goes and the curling ceases; rapid plant growth then takes place. The subsequent growth is finally proportional to the amount of food present.

5. Plants grown in soils heated to  $100^{\circ}$  show a very remarkable development of fibrous root unlike anything obtained on untreated soils.

6. Plants grown on soils heated to  $100^{\circ}$  have, in comparison with those on untreated soils, larger leaves of deeper green colour, stouter stems, usually shorter internodes; they flower earlier and more abundantly, and contain a higher percentage of nitrogen and sometimes of phosphoric acid in their dry matter; the roots and stems give up their nitrogen, phosphorus, and potassium more completely to the fruit.

7. Plants grown on soils heated to  $55^{\circ}$  or treated with volatile antiseptics show fewer of these effects; there is only rarely a retardation in seedling growth but usually an acceleration, sometimes a rapid one, succeeded by a period of steady growth which is finally proportional to the amount of plant food present. No specially marked development of fibrous root or shortening of the internodes occurs, but there is an increase in the percentage of nitrogen and sometimes of phosphoric acid in the dry matter as compared with plants raised on untreated soils, and also a more complete translocation of these materials to the fruit.

Two of these effects are confined to soils heated to  $100^{\circ}$ , viz. the marked development of fibrous root, and the shortening of the internodes.

The other effects are more general, viz. alteration (either retardation or acceleration) in the rate of germination and early growth; steady growth of the older plant proportionately to the amount of plant food produced in the soil; earlier and more prolific flowering and fruiting (Figs. 1 and 7); increase in the percentage of nitrogen and phosphoric acid in the dry matter, and more complete translocation of these constituents and of potassium from the root and stems to the fruit.

We may now turn to the chemical differences in the soils.



Partially sterilised soils are characterised by the fact that ammonia accumulates unchanged and nitrates are no longer formed (the nitrifying organisms being killed). Untreated soils contain practically no

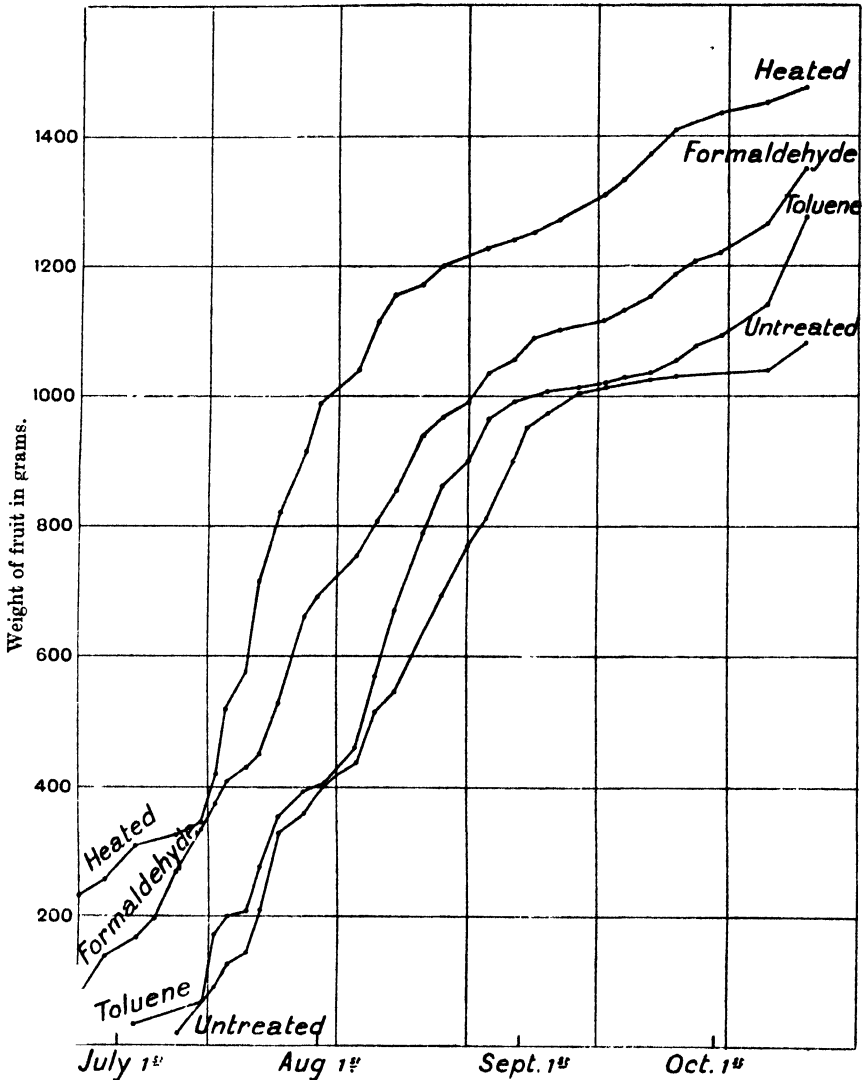


Fig. 1. Showing the rate of fruiting of tomatoes in variously treated soils.

ammonia. As the decomposition processes are all accelerated, ammonia and its antecedent bodies are present in partially sterilised soils in greater amount than the nitrates and antecedent bodies in

untreated soils. Thus the aqueous extract of partially sterilised soils is richer in nitrogen, in ammonia, and in the so-called "albuminoid ammonia" than that of untreated soils.

Soils heated to 100° C. are further characterised by the presence of decomposition products, some of which are soluble and give a brown colour to an aqueous extract, while others have a peculiar odour recalling that of liquorice. Some of the products are specially suited for the nutrition of certain moulds<sup>1</sup>, especially *Pyronema glaucum* which often developes to a remarkable extent; and others are very unsuited for the development of certain bacteria<sup>2</sup>. None of these products has yet been identified in our soils<sup>3</sup>. Soils that have been heated to 100° appear to become more moist than untreated soils when both receive the same amount of water.

### *Discussion of the observations.*

Several hypotheses have been put forward to account for these results, at least two of which are of considerable importance.

*Germination.* S. U. Pickering was the first to observe that germination is retarded in partially sterilised soils. His work arose out of an observation that young fruit trees started much more slowly into growth in heated than in untreated soils<sup>4</sup>, although later on they made considerably better growth. As this particular problem appeared likely to require a long while for investigation he began by working on germination.

In his first paper, published in 1908<sup>5</sup>, he states that germination is adversely affected in soil which has been heated without drying to temperatures from 60° to 150°; the total number of seeds that germinate decreases in most cases, and the time necessary for germination increases, with the temperature of heating. The result is not due to any change

<sup>1</sup> This fact is curiously overlooked by mycologists. We read, for instance, in a recent paper by a well-known mycologist that a fungus known to attack the roots of certain plants was inoculated into soil heated above 100° C. in order to ascertain whether it could live and thrive in a soil free from living plants. Good growth was obtained, as we should expect, but the wholly erroneous conclusion was drawn that the fungus "is able to live in, and to penetrate for some distance into, the ordinary soil in our fields."

<sup>2</sup> This *Journal*, 1909, **3**, 135, and 1912, **5**, 200.

<sup>3</sup> For an account of the American work on this subject see Schreiner and Lathrop, "The chemistry of steam heated soils," *Bul.* 89, *Bureau of Soils*, 1912.

<sup>4</sup> *Nature*, June 6th, 1907.

<sup>5</sup> "Studies in germination and plant growth." This *Journal*, 1908, **2**, 411—434.

in the bacterial flora<sup>1</sup>. An increase in the soluble constituents of the soil, especially the organic matter and soluble nitrogen compounds, was found to result from the heating, and the increase was considered to be directly proportional to the increase in time required for germination. "The latter increase appears, therefore, to be due to the formation of a nitrogenous compound in the soil, which is inhibitory towards germination." The premises were subsequently modified somewhat, the direct proportion being found not to hold<sup>2</sup>, he therefore supposes "either that the inhibiting substance produced by heating different soils is not the same in all cases, or, more probably, that it constitutes only one of the organic products formed when a soil is heated, the proportions between it and the other non-inhibiting substances formed varying in each particular case."

In this second paper he showed that soils treated with antiseptics also adversely affected germination and behaved like soils heated to 60°—75°. He further states that "soils in their natural state appear generally to contain a certain amount of this inhibitory substance."

A third paper<sup>3</sup> is devoted to a study of the properties of the soluble matter in soils, and the conclusion is drawn that the amount of soluble organic matter, and the toxic qualities of heated soils, are reduced on storing the soils under moist, aerated conditions.

There is no doubt that heat causes decomposition in the soil, the amount of which increases as the temperature of heating rises. Pickering inclines to the belief that the change is regular and progressive: our own experiments, however, indicate that the decomposition going on above 80° is far more serious than that taking place at lower temperatures. It is equally certain that something is produced harmful to germination and, as we have shown elsewhere (see p. 255), to bacterial activity. Since the water extracts of the variously treated soils cause accelerations and retardations in germination of the same order as is shown by the soils themselves it follows that the active substances are partly, at any rate, soluble in water.

<sup>1</sup> These observations were made on *Lolium perenne*, *L. italicum*, clover and spinach, and are of interest in connection with a hypothesis, attributed to Nilson, that germination depends on the activity of bacteria at the surface of the seed. W. Windisch and K. Schönewald (*Woch. Brau.* 1905, **22**, 200) showed that this was not true of barley, and Dixon (*Trans. Roy. Soc. Dub.* v. **11**, 1) that it did not hold for turnips. Hutchinson and Miller have also germinated peas and wheat in absence of bacteria (*This Journal*, 1912, **4**, 282—302). R. W. Stigell has discussed changes whereby bacteria may affect germination and subsequent plant growth (*Centr. Bakt. Par.* 1909, **28**, 727).

<sup>2</sup> "The action of heat and antiseptics on soils." *This Journal*, 1908, **3**, 32—54.

<sup>3</sup> "Studies of the changes occurring in heated soils." *This Journal*, 1910, **3**, 258—276.

But there is no evidence that these active substances are necessarily organic. Even if the supposed proportionality between the amount of soluble nitrogen and organic matter on the one hand, and the retardation of germination on the other, had been confirmed, the proof is inconclusive because the conditions would be fulfilled by ammonia just as well as by organic matter. Now that the proportionality is found not to be general the argument in favour of the invariable organic nature of the retarding agent loses its force.

Unfortunately the soil extract, like the soil itself, is very complex in composition and not readily resolved into its constituents, so that recourse cannot be had to the simple expedient of trying the effect of each constituent on germination. It is certain, however, that ammonia and nitrates are present, and we have made some experiments to ascertain what part they play in the matter.

Very dilute solutions of ammonium hydrate (one part per million of the medium) were found to accelerate germination under the conditions of our experiments. Stronger solutions had no effect, still stronger solutions retarded the process, and finally at higher concentrations germination was prevented altogether. The details of the effect vary considerably with the conditions, but the experiments show that when, for any reason, germination is slow, very dilute ammonia solutions tend to accelerate it; when germination is more rapid, ammonia tends to retard it.

This result is probably connected with another obtained by A. J. Brown<sup>1</sup>. If barley seed is placed in solutions of various substances, water alone enters the seed and not the dissolved substance as a rule. There are, however, certain substances that can penetrate the membrane<sup>2</sup>, among them ammonia, and in this case water enters the seed more rapidly from the solution than from pure water. It seems likely that this increased speed of entry of the water into the seed is in part responsible for the acceleration induced by very dilute ammonia solutions in the germination of slowly germinating seeds. The fact also that ammonia enters the seed probably accounts for its very drastic effects at greater concentrations.

Ammonium sulphate solutions are much weaker in their action on germination and sodium nitrate solutions weaker still at equivalent concentrations.

<sup>1</sup> *Proc. Roy. Soc.* 1909, **81** B, 82—93. The selective permeability of the coverings of the seeds of *Hordeum vulgare*.

<sup>2</sup> These are the "hormones" of H. E. and E. F. Armstrong, see p. 260.

It may be taken as certain that all the soluble constituents of the soil have an effect on germination. They probably fall into at least two groups, some behaving like ammonia and causing very great retardation at higher concentrations, while others like sodium nitrate have much less effect. Further experiments would be necessary to decide whether all the powerful retarders would accelerate in dilute solutions as ammonia does. F. J. Seaver and E. D. Clark<sup>1</sup> have argued that the decomposition products are not actually toxic to higher plants, but are harmful only by reason of their excess. They make the important point that, as *Pyronema* and other moulds will grow on heated soils more readily than on unheated soils, it is obviously unsound to speak generally of toxins in heated soils. But so long as we confine ourselves to the higher plants we think we are safe in making the distinction given above.

Our experiments indicate that germination is too sensitive a process, and too susceptible to external influences, to afford much help in studying the soil. It is greatly affected by small changes in moisture content, ammonia content, temperature, etc., and is more useful in detecting such changes than in studying them. We do not, therefore, agree that the retardation of germination affords any proof of the formation of any particular organic toxin, but consider that it may result from any change in the soluble constituents; some substances, including ammonia and doubtless certain organic compounds, have a very marked effect, while others, such as nitrates, have a smaller action.

This conclusion also harmonises with most of Pickering's experimental results. The main discrepancies between his results and ours are that (1) we find accelerated germination in certain cases in soils heated to 55°, (2) we failed to trace any proportionality between the analytical data for the soil and the amount of retardation or acceleration in germination<sup>2</sup>. The effect produced depended so much on the moisture, the temperature, and other conditions of the experiments, and also on the individuality of the seeds, that we could not express it by any number; nor could we even find any general connection between the nature of the effect and the amount of nitrate or ammonia in the soil.

<sup>1</sup> "Biochemical studies on soils subjected to dry heat," *Biochem. Bull.* 1912, **1**, 413-427.

<sup>2</sup> It should be noted that we are not dealing with highly heated soils as used in some of Pickering's experiments but confined ourselves to soils treated with antiseptics or heated to 100° C. or less. When the temperature of heating rises above 100° the decomposition proceeds much more rapidly and the effect on the plant is correspondingly more drastic.

Lastly, we could obtain no definite proof that the harmful effect of heated soil on germinating seeds passes off after a time. The difference in behaviour between soil heated to 100° and untreated soil apparently becomes reduced on storage, but the untreated soil itself was found to change in composition and behaviour towards germinating seeds. No unchanging standard could be discovered for making any strict comparison between freshly treated and stored soils.

*The seedling stage and early growth.* Some interesting physiological problems are presented by the subsequent effects shown during the seedling stage and up to the formation of the third or fourth leaves. The curling of the leaf towards the under side, the very dark green colour and the purple patches all tend apparently to reduce the amount of assimilation, but it is not at all obvious why this should be necessary to plants growing in treated soil where there is so much plant food present. Somewhat similar phenomena can be induced by restricting the supply of potassium and giving excess of nitrogen; the young plants in several ways resemble the mangolds on Plot 5 AC of Barnfield where large dressings of ammonium sulphate and rape cake are given, but no potassium salts. (In this case there is no purple colour but the leaf stems are orange instead of the normal pale green.) Further, the effect does not depend entirely on any soil constituent, but requires also certain external conditions not definitely ascertained, of which poor light appears to be one.

For the present we prefer not to discuss the immediate causes of these phenomena but to confine ourselves to another remarkable relationship: the close connection between the pigmented or stunted leaves and small growth on the one hand, and the large green leaf and rapid growth on the other. As already stated, we can never be quite certain how *young* plants will behave in partially sterilised soils; they may either grow much more rapidly than those on untreated soils, throwing out larger cotyledon leaves and larger subsequent leaves, or they may show the remarkable pigmented or curling effects and considerable retardation of growth. These two sets of phenomena are very closely connected and it would be quite reasonable to suppose that they are both produced by the same factors, either retardation or acceleration setting in according as some small change just shifts the balance one way or the other.

It was at one time considered that any substance toxic to plants may act as a stimulant if supplied in sufficiently minute quantities, and on this view it is only necessary to suppose that partial sterilisation

leads to the formation of a toxin which is sometimes in such small amounts that it stimulates and sometimes in larger amounts so that it retards growth. But it is now known that so typical a toxin as copper sulphate retards growth and never increases it in any well conducted experiment. This view must therefore be regarded as too vague to be helpful. A much more definite conception has been put forward by H. E. and E. F. Armstrong<sup>1</sup>. Certain substances which usually have but a slight attraction for water are capable of penetrating plant membranes and entering the cells: instances are toluene, carbon disulphide, chloroform, etc. Once there they are considered to stimulate enzymic activity, and exercise a determining influence in regulating metabolism; one effect, for instance, is to condition the introduction of water which dilutes the cell contents and thus determines the occurrence of downgrade changes. The authors further suggest that the absorption of salts by plants, and the translocation of diffusible material, may be largely determined by some such process. To these bodies the name hormone is given; they are not a purely arbitrary group but possess certain chemical and physical properties which more or less differentiate them from others.

Now ammonia occurs in the list of hormones and is described as acting very rapidly. We have seen that it behaves in a special manner towards the germination of seeds, accelerating the process in very dilute solutions (the typical hormone action) and considerably retarding it in rather stronger solutions, the action being very much more marked than that shown by solutions of salts, even of ammonium sulphate. Ammonia also occurs in all the soils, especially in those that have been partially sterilised. Unfortunately we have no means of knowing precisely in what state it occurs, but some at any rate is apparently free. In view of its special behaviour towards the cell membranes we have no right to regard it solely as a nutrient but must consider the possibility of other effects. It would not be difficult to sketch out a hypothesis to account for the retardation and acceleration results on the assumption that the ammonia in the soil acts as a hormone.

Our own data are insufficient to settle the problem because the partially sterilised soil differs in another way from the untreated soil; it contains a different set of nutrients; there are thus two variables.

Pickering has shown that soils heated to 125° and 130° C.<sup>2</sup> are

<sup>1</sup> "The origin of Osmotic effects, III." *Proc. Roy. Soc.* 1910, **82** B, 588—602. "The function of hormones in regulating metabolism." *Annals of Botany*, 1911, **25**, 507—519.

<sup>2</sup> "Plant growth in heated soils." *This Journal*, 1910, **3**, 277—284.

less favourable to the growth of spinach, tomato, and tobacco than soils heated to 100° C. only, and concludes that at the higher temperature a larger production of toxin has taken place. We have repeated the experiment with tomatoes and obtained a like result. Since the second crop is not adversely affected he supposes that the toxin is unstable and gradually disappears by the action of air and moisture. The supposition is unnecessary: in so far as ammonia is responsible for the retardation its effect in a cropped soil must steadily diminish as it is absorbed by the plant. A second crop in any case is less likely to suffer than an early sown first crop: all our observations show that the retarding effects are less marked in summer than in early spring.

The marked development of fibrous root in the soil heated to 100° C. is being further studied; it occurs in a top dressing of heated soil added to a border of untreated soil, and such top dressings are being now adopted to induce fine root growth in one or two nurseries where some of our experiments have been made.

The difference in composition in dry matter—an increased percentage of nitrogen and sometimes of phosphoric acid in the case of plants grown on partially sterilised soils—seems to be associated with the differences set up in the soil. Plants fed on ammonium salts under conditions where nitrification is suspended commonly contain a higher percentage of nitrogen than plants fed in the normal manner on nitrates. The phosphoric acid relationships are being further studied. But it is probable that the altered composition of the leaves and stems and more complete translocation of nitrogen, phosphorus and potassium to the fruit are closely connected with the earlier and more prolific fruiting and with the change in quality of the fruit.

No hypothesis has yet been put forward to account for the shortening of the internodes in the plants grown in the heated soil.

## EXPERIMENTAL PART.

### *Germination.*

*The effect of soil treatment on the rate of germination.* The soil was passed through a 3 mm. sieve and then carefully divided into several similar portions each of which was treated in its proper manner. One was heated to 100° C. for three hours in a steam oven; another received 0.5 per cent. of toluene which at the end of 24 or 30 hours was allowed



to evaporate by spreading the soil in a thin layer, the water simultaneously lost being subsequently added; the third was heated to 55° C. for three hours in a water oven; while the fourth was left untreated. In order to study the behaviour of germinating seeds 40 gram lots of the treated and untreated soils were weighed into Petri dishes, there being usually six dishes of each of the various soils, *i.e.* 24 altogether. The same amount of water was added to each dish so that all should be uniformly moist; the soil was made distinctly wet but was not actually waterlogged. One hundred seeds (usually turnips or tomatoes) were placed on the top of the soil in each dish but not buried, and the whole of the dishes were then put into the incubator at 22° C. After germination had begun the dishes were taken from the incubator so that the number of germinated seeds might be counted. Any seed in which the radicle could be seen was regarded as having germinated. To facilitate the counting, the obviously germinated seeds were removed by an assistant from each dish and set aside, and the more doubtful ones were then examined carefully by one of us. With practice it was found possible to get through all the dishes fairly quickly.

TABLE I. *Number of seeds germinated in given periods.*  
*Turnip seed. Temperature 18° C.*

No. of dish	15 hrs.	17 hrs.	19 hrs.	21½ hrs.	26½ hrs.	39 hrs.	63 hrs.	87 hrs.	3 days later
1	6	16	46	73	83	89	92	92	98
2	5	21	46	67	78	90	95	96	100
3	5	18	45	69	80	91	95	96	99
4	5	18	44	71	81	93	98	101	105
5	6	23	54	75	81	93	99	100	102
6	6	21	50	69	78	88	92	93	100

Thus each unit in our experiment consists as a rule in six dishes of 100 seeds each, *i.e.* 600 seeds altogether. By using this large number we minimise the difficulties due to the very considerable individual variation in seeds, and reduce our experimental error to comparatively low proportions. Table I gives the detailed counts for the six untreated soils in one experiment, the probable error for the individual dishes is 1 to 2.5, and the mean error for the six is about 1. The errors for the treated soils are of the same order.

TABLE II. *Number of seeds germinating in given periods in various soils immediately after treatment.*

A. *Poor arable soil.* "Little Hoos" containing 0.12% N, 2.10% CaCO<sub>3</sub> and losing 5.31% on ignition.

Turnip seed. 28. III. 11.

Soil treatment	Ammonia and Nitrate per million of dry soil			Seeds germinated after					Total germinating
	Ammonia	Nitrate	Total	19 hrs.	24 hrs.	27 hrs.	39 hrs.	63 hrs.	
Untreated .....	1	9	10	6	44	306	435	463	497
Heated to 100° C. ....	4	9	13	4	61	309	421	456	490
" " 55° C. ....	1	9	10	8	58	315	437	471	503
Treated with Toluene ..	2	9	11	2	33	294	420	462	501

Exp. 115, 47.

B. *Arable soil.* "Knotwood" much richer in nitrates and containing 0.18% N, 0.37% CaCO<sub>3</sub>, and losing 7.6% on ignition.

Turnip seed. Moisture=36%. 29. XI. 11.

Soil treatment	Ammonia and Nitrate per million of dry soil			Seeds germinated after				Total germinating
	Ammonia	Nitrate	Total	18½ hrs.	22½ hrs.	39½ hrs.	64½ hrs.	
Untreated .....	5	56	61	89	291	461	512	544
Heated to 100° C. ....	8	56	64	83	271	486	547	574
" " 80° C. ....	7	56	63	78	262	465	519	553
" " 55° C. ....	5	56	61	77	277	463	524	561

Exp. 143, 13.

(With less water there had been acceleration at 55° C., no action at 80° C., and retardation at 100° C.)

C. *The same soil.* Tomato seed. Moisture=33%.

Soil treatment	Seeds germinated after			
	46 hrs.	70 hrs.	95 hrs.	
Untreated .....	70	106	132	340 seeds in each set
Heated to 100° C. ....	55	90	109	
" " 80° C. ....	60	77	94	
" " 55° C. ....	62	94	115	

Exp. 143, 17.

TABLE II (cont.).

D. Garden soil containing 0.42% N, 2.6% CaCO<sub>3</sub> and losing 12.8% on ignition.  
Turnip seed. Moisture=40.7%. 20. VII. 11. Temp. 25°C.

Soil treatment	Seeds germinated after						Total germinating
	15 hrs.	17 hrs.	18½ hrs.	21 hrs.	23 hrs.	39 hrs.	
Untreated.....	49	195	303	435	460	560	592
" ..	48	197	309	442	472	568	598
Heated to 100°C....	62	211	318	409	452	552	592
" .. 55°C....	63	246	358	445	473	564	586

Exp. 115, 94.

E. Turnip seed. The same soil. Moisture=36%. 6. XII. 11.

Soil treatment	Seeds germinated after					Total germinating
	17½ hrs.	19 hrs.	23 hrs.	40 hrs.	47½ hrs.	
Untreated .....	51	169	369	528	542	591
Heated to 100°C.	23	128	355	519	532	595
" .. 80°C.	57	180	380	539	556	615
" .. 55°C.	89	198	375	515	528	589

Exp. 143, 21.

F. Tomato seed. Same soil. Moisture=39%. 9. XII. 11.

Soil treatment	Seeds germinated after				Total germinating
	45 hrs.	53 hrs.	69 hrs.	76 hrs.	
Untreated .....	250	367	462	503	554
Heated to 100°C.	74	136	336	443	528
" .. 80°C.	92	149	323	463	527
" .. 55°C.	106	165	360	465	534

Exp. 143, 25.

G. Very rich greenhouse soil. "Ox. L." containing 0.63% N, 1.93% CaCO<sub>3</sub> and losing 16.9% on ignition.

Turnip seed. Moisture=60%. 14. XII. 11.

Soil treatment	Ammonia and Nitrate per million of dry soil			Seeds germinated after						Total germinating
	Ammonia	Nitrate	Total	16½ hrs.	19 hrs.	23½ hrs.	40 hrs.	64 hrs.	88 hrs.	
Untreated .....	14	360	374	18	106	361	521	562	566	608
Heated to 100°C. ..	60	360	420	6	86	343	514	557	563	597
" .. 80°C. ....				11	76	324	504	555	560	598
" .. 55°C. ....				18	121	382	522	561	564	601

Exp. 143, 29.

TABLE II (*cont.*).

H. Tomato seed. Same soil. Moisture=60%. 19. XII. 11.

Soil treatment	Seeds germinated after		
	46½ hrs.	53 hrs.	
Untreated .....	124	202	340 seeds in each set
Heated to 100°C. <sup>1</sup>	24	44	
" " 80°C.	21	42	
" " 55°C.	84	137	

Exp. 143, 33.

The moisture is in all cases calculated on the moist soil and the percentage composition on the dry soil.

<sup>1</sup> In this case the soil after heating to 100° wetted more easily than the untreated soil. This is the reverse of what usually happened.

TABLE III. *Effect of storing the soil after treatment. Number of seeds germinating in given periods.*A. Greenhouse soil. "R.C." (tomato soil) containing 0.37% N, 0.57% CaCO<sub>3</sub> and losing 8.7% on ignition. Turnip seed.

1. Immediately after treatment. Moisture=47.5%. 12. VII. 11. Temp. 18° C.

Soil treatment	Ammonia and Nitrate per million of dry soil			Seeds germinated after						Total germinating
	Ammonia	Nitrate	Total	16 hrs.	18 hrs.	20 hrs.	22½ hrs.	27½ hrs.	40 hrs.	
Untreated .....	10	50	60	33	117	285	424	481	544	602
Heated to 100°C.	38.5	50	88.5	18	99	247	380	467	530	590
" " 55°C.	23	50	73	23	119	292	428	489	556	602
Treated with Toluene	30.5	50	80.5	27	109	270	391	460	536	595

Exp. 115, 88.

2. Two months after treatment. Moisture=47.5%. 12. IX. 11. Temp. 20° C.

Soil treatment	Ammonia and Nitrate per million of dry soil			Seeds germinated after					Total germinating
	Ammonia	Nitrate	Total	14 hrs.	16½ hrs.	19½ hrs.	22½ hrs.	26 hrs.	
Untreated .....	7	149	156	47	196	342	424	511	543
Heated to 100°C.	85	(50)	135	36	190	345	429	532	572
" " 55°C.	7	147	154	51	202	357	430	518	552
Treated with Toluene	71	95	166	59	208	358	433	517	560

Exp. 135, 8.

TABLE III (*cont.*).

3. Six months after treatment. Moisture=47.5%. 9. I. 12. Temp. 17° C.

Soil treatment	Ammonia and Nitrate per million of dry soil			Seeds germinated after				Total germinating
	Ammonia	Nitrate	Total	18 hrs.	21½ hrs.	39 hrs.	63 hrs.	
Untreated .....	4	176	180	34	212	484	517	540
Heated to 100° C. ....	99	50	149	33	200	493	547	566
" " 55° C. ....	5	167	172	36	204	509	544	559
Treated with Toluene	90	83	173	24	216	483	614	629

Exp. 135, 52.

(For results with sand containing equivalent amounts of  $(\text{NH}_4)_2\text{SO}_4$  and  $\text{NaNO}_3$  see Table VI, D.)

B. Tomato seed. Immediately after treatment. Same soil as for turnips in all cases. 15. VII. 11. Temp. 21° C.

Soil treatment	Seeds germinated after									Total germinating
	40 hrs.	46 hrs.	50 hrs.	63 hrs.	71 hrs.	87 hrs.	95 hrs.	111 hrs.	135 hrs.	
Untreated .....	18	44	71	164	205	219	254	275	285	288
Heated to 100° C. ....	2	3	7	40	65	142	154	213	266	300
" " 55° C. ....	1	4	15	70	108	187	202	246	279	295
Treated with Toluene	0	0	0	33	57	132	154	205	272	290

Exp. 115, 91.

Six months after treatment. Same soil as for turnips. 9. I. 12.

Soil treatment	Seeds germinated after			Total germinating
	44 hrs.	49 hrs.	68 hrs.	
Untreated .....	158	297	390	400
Heated to 100° C. ....	69	194	385	407
" " 55° C. ....	80	218	387	401
Treated with Toluene	82	222	385	402

Exp. 135, 62.

TABLE III (cont.).

C. Garden soil containing 0.42% N, 2.6% CaCO<sub>3</sub> and losing 12.8% on ignition.

• Turnip seed. Immediately after soil treatment. Moisture = 36%.

14. IX. 11. Temp. 22° C.

Soil treatment	Ammonia and Nitrate per million of dry soil			Seeds germinated after				Total germinating
	Ammonia	Nitrate	Total	15½ hrs.	17½ hrs.	19½ hrs.	22½ hrs.	
Untreated .....	6	44	50	43	137	290	391	572
Heated to 100° C. ..	22	47	69	52	147	293	402	577
" " 55° C. ....	15	44	59	92	214	331	415	582
Treated with Toluene	15	41	56	75	181	315	393	563

Exp. 135, 11.

This soil had been kept in bottles for two months previous to treatment.

Four months after treatment. Moisture = 40%. 16. I. 12. Turnip seed.

Soil treatment	Ammonia and Nitrate per million of dry soil			Seeds germinated after			Total germinating
	Ammonia	Nitrate	Total	18 hrs.	21 hrs.	42 hrs.	
Untreated .....	8	82	90	89	202	513	553
Heated to 100° C. ..	87	78	165	82	262	525	568
" " 55° C. ....	23	105	128	80	238	525	570
Treated with Toluene	9	120	129	80	233	526	554
Sand and distilled water .....				167	314	506	558

Exp. 135, 58.

Arable soil containing 0.11% N, 3.4% CaCO<sub>3</sub> and losing 4.0% on ignition.

4½ years after treatment. 11. I. 12. Turnip seed. Moisture = 40%.

Soil treatment	Seeds germinated after				Total germinating
	17½ hrs.	20 hrs.	23 hrs.	41½ hrs.	
Untreated .....	15	66	140	199	225
Heated to 100° C. ....	8	52	113	187	213
Treated with Toluene	12	47	121	203	224

Exp. 135, 54.

TABLE IV. *Effect of varying soil moisture content on the rate of germination of seeds.*

A. Arable soil ("Knotwood field") containing 0.18% N, 0.37%  $\text{CaCO}_3$  and losing 7.6% on ignition. Turnip seed. 13. III. 12.

Soil treatment	Ammonia and Nitrate per million of dry soil				Seeds germinated after						Total germinating
	Ammonia	Nitrate	Total		16 hrs.	18½ hrs.	21 hrs.	23½ hrs.	40 hrs.	64 hrs.	
Untreated	2	24	26	Lower moisture	11	48	172	265	480	534	545
				Higher "	28	91	212	308	508	546	560
Heated to 100° C.	11	23	34	Lower moisture	3	35	135	229	486	556	580
				Higher "	18	55	157	256	463	526	545
Heated to 58° C.	10	24	34	Lower moisture	20	70	199	306	512	545	564
				Higher "	29	100	218	312	487	533	545
Treated with Toluene	8	23	31	Lower moisture	5	41	151	241	474	534	558
				Higher "	11	51	165	254	483	543	557
Sand				Lower moisture	13	38	136	209	470	527	537
				Higher "	41	119	234	327	487	529	550

Exp. 158, 1.

Same soil. Tomato Seed.

Soil treatment		Seeds germinated after					Total germinating
		40 hrs.	45 hrs.	64 hrs.	72 hrs.	88 hrs.	
Untreated soil .....	Lower moisture	249	303	458	462	468	480
	Higher "	228	300	467	474	482	493
Heated to 100° C. .	Lower moisture	79	106	318	390	441	474
	Higher "	36	58	261	324	434	480
" " 55° C. .	Lower moisture	126	166	400	430	459	483
	Higher "	113	147	380	428	465	480
Treated with Toluene	Lower moisture	101	144	361	414	451	479
	Higher "	94	149	344	387	445	474
Sand .....	Lower moisture	18	35	266	364	452	477
	Higher "	58	97	332	410	467	488

Exp. 158, 9.

In each case the lower moisture = 31.6% and the higher moisture = 37.5%.

TABLE IV (*cont.*).

B. The same soil stored for eight weeks. Turnip seed. 21. V. 12.

Soil treatment	Ammonia and Nitrate per million of dry soil				Seeds germinated after					Total germinating
	Ammonia	Nitrate	Total		16 hrs.	18 hrs.	20 hrs.	23 hrs.	40 hrs.	
Untreated soil	2	27	29	Lower moisture	31	67	128	214	472	518
				Higher "	31	80	149	249	495	548
Heated to 100° C.	44	22	66	Lower moisture	39	76	164	268	489	559
				Higher "	21	69	134	245	482	559
Heated to 55° C.	41	25	66	Lower moisture	44	92	181	272	425	510
				Higher "	48	131	211	311	505	572
Treated with Toluene	45	26	71	Lower moisture	28	75	134	235	433	518
				Higher "	36	107	191	302	510	555
Sand				Lower moisture	38	104	167	237	513	572
				Higher "	86	169	239	318	480	462

Exp. 158, 36.

In each case the lower moisture=31.6% and the higher moisture=37.5%.

C. A rich greenhouse soil containing 0.63% N, 1.93% CaCO<sub>3</sub> and losing 16.9% on ignition. Turnip seed. 30. IV. 12.

Soil treatment	Ammonia and Nitrate per million of soil				Seeds germinated after						Total germinating
	Ammonia	Nitrate	Total		16 hrs.	18½ hrs.	21½ hrs.	24 hrs.	40 hrs.	64 hrs.	
Untreated soil	13	315	328	Lower moisture		113	302	417	518	542	553
				Higher "	34	122	277	390	511	535	545
Heated to 100° C.	64	323	387	Lower moisture		47	241	324	469	503	523
				Higher "	12	35	115	240	473	520	551
Heated to 55° C.	—	—	—	Lower moisture		89	246	338	497	525	538
				Higher "	27	112	281	381	506	550	568
Treated with Toluene	23	295	318	Lower moisture		70	261	363	520	549	556
				Higher "	10	80	172	291	478	513	542
Sand	—	—	—	Lower moisture		52	190	310	516	542	553
				Higher "	32	110	265	359	488	528	554

Exp. 158, 27.



TABLE IV (*cont.*).

The same soil. Tomato seed. 1. V. 12.

Soil treatment		Seeds germinated after					Total germinating
		40 hrs.	15 hrs.	18 hrs.	64 hrs.	88 hrs.	
Soil untreated. . .	Lower moisture	59	140	196	407	451	479
	Higher „	79	212	269	420	454	479
Heated to 100° C. ...	Lower moisture	2	4	8	66	189	480
	Higher „	1	4	4	47	197	508
Heated to 55° C. ...	Lower moisture	13	27	43	180	320	478
	Higher „	17	32	48	222	347	491
Treated with Toluene	Lower moisture	5	20	41	191	341	479
	Higher „	4	15	22	190	370	486
Sand . . . . .	Lower moisture	—	3	12	226	416	479
	Higher „	31	72	105	327	433	472

Exp. 158, 31.

In each case the lower moisture=45% and the higher moisture=52%.

The varying effects of partially sterilised soil on germination are shown in Tables II, III and IV. In a certain number of cases germination is most rapid in the untreated soil, less rapid in the soil heated to 55°, still less in the soil treated with the toluene, and slowest in the soil heated to 100°. The amounts of ammonia in the soil run in the same direction as the extent of the retardation and in these instances it might be supposed that the retardation was approximately proportional to the amount of ammonia present. But there are too many exceptions to allow of this assumption; many cases occur among the poorer soils where germination takes place most rapidly in the soil heated to 55°, and in two or three instances germination is more rapid in the soils heated to 100° or treated with toluene than in the untreated soil. This is with turnip seed; with tomato seed different results are obtained, the order of the soils being practically always as follows: untreated soil (germination most rapid); soil heated to 55°, to 84° or treated with toluene; soil heated to 100° (germination slowest).

*The effect of storage.* When soils have been stored for some time after partial sterilisation they undergo a change in composition shown by an increase in the total ammonia and nitrates. The increase is most marked in the soil heated to 100° where it takes the form of

ammonia, less marked in the soil treated with toluene where it appears either as ammonia or nitrates, and sometimes still less marked in the soil heated to 55°, but is least in the untreated soil where it occurs only in the nitrates.

The effect on germination is shown in Table III. Of the stored soils those that have been heated to 55° or treated with toluene are generally more favourable or less unfavourable to germination than those heated to 100°, but not invariably. The effect does not pass off with time, but no estimate can be formed as to how much it changes because the untreated soil alters also. There is in fact no unchanging standard whereby measurements could be taken. Further complication arises from the fact that the phenomena depend to some extent on the amount of water present in the soil.

*The effect of varying moisture content.* As the amount of moisture in the soil is increased so the retarding effect of the soil heated to 100° becomes more marked. This was demonstrated in the following experiment. Tomatoes were sown in a rich soil (a pasture soil) but insufficient water was added for rapid germination: periodical counts showed that only a slight retardation was produced in the soil heated to 100°. On adding more water germination became much more rapid, but the inhibitory effect of the heated soil now manifested itself.

The results were:

Soil treatment	1st Period, insufficient moisture			2nd Period, more water added		Total germinating
	Seeds germinated after			Seeds germinated after		
	64 hrs.	88 hrs.	114 hrs.	136 hrs.	160 hrs.	
Untreated.. .. .	8	18	31	70	113	119
Heated to 100° C.	3	15	31	52	93	115
"  "  55° C.	8	21	30	65	112	118

21. III. 11. Exp. 115, 45.

More complete evidence is given in Table IV. Soil heated to 55° or treated with toluene generally shows the same phenomena. Similar results were obtained with sand watered with dilute ammonia solution (Table VI), so that the effect does not depend entirely on any property of the soil.

*The effect of altered physical conditions.* While carrying out the experiments it became evident that some physical change took place on partial sterilisation because the treated and untreated soils showed considerable difference in their behaviour when watered, as already noted by Pickering<sup>1</sup>. Water readily soaked into the untreated soils, but it stood in drops on the others and did not penetrate for some time, especially into the soil heated to 100°. When all the soils received the same weight of water there was an obvious difference in their appearance, the treated soils appearing to be the wettest. This physical difference does not appear to be the determining factor in bringing about the germination phenomena for the following reasons:

1. In all the experiments in Table II there was excess of water.
2. Where less water was used the difference between the untreated and the treated soils did not become more marked but less (Table IV).
3. An aqueous extract of the soil made by stirring up the soil with twice its weight of water and filtering through a Buchner funnel also affected the rate of germination.

*The behaviour of the soil extract.* Extracts made in the manner stated above were added to quartz sand (40 grams) contained in Petri dishes. Seed was then sown and the experiment was conducted in the usual manner. The results are given in Table V: they show the same kind of differences as were obtained in the experiments with soils, the extract of the soil heated to 55° sometimes accelerating and sometimes retarding germination in comparison with the extract of untreated soil, while the extract of the soil heated to 100° has a retarding effect. In two cases out of the three the soil extracts all retarded germination

TABLE V. *Effect of aqueous extracts of soils on germination.*

A. Extract of greenhouse soil R.C. Turnip Seed. 19. X. 11.					
Soil treatment	Seeds germinated after				Total germinating
	16 hrs.	19 hrs.	22½ hrs.	38½ hrs.	
Untreated .....	84	266	373	500	598
Heated to 100° C. ....	83	255	381	496	596
" " 55° C. ....	93	286	382	493	592
Treated with Toluene	78	280	374	494	596

Exp. 135, 22.

<sup>1</sup> This *Journal*, 1910, 3, 261.

TABLE V (*cont.*).

B<sup>1</sup>. Extract of garden soil as used in Table III, B. Turnip seed. Temp. 19° C.  
15. IX. 11.

Soil treatment	Seeds germinated after				Total germinating
	19½ hrs.	21½ hrs.	25 hrs.	37 hrs.	
Untreated .....	36	124	334	558	568
Heated to 100° C. ....	18	84	297	576	581
" " 55° C. ....	26	106	319	561	571
Treated with Toluene ..	17	84	305	567	580
Distilled water .....	23	90	290	557	568

Exp. 135, 12.

<sup>1</sup> In this experiment the temperature fell to 14° during the first 12 hours, it was then put up to 23°. A 20% extract was used in this case.

The data for the soil are given in Table II, B.

C. Extract of pasture soil. Turnip seed. 20. II. 12.

Soil treatment	Seeds germinated after					Total germinating
	17 hrs.	19 hrs.	22 hrs.	24½ hrs.	41 hrs.	
Untreated .....	29	58	177	306	497	559
Heated to 100° C. ....	20	49	147	265	473	564
" " 59° C. ....	24	64	183	308	502	573
Distilled water .....	36	118	253	366	508	576

Exp. 135, 72.

D. Extract of the same soil. Tomato seed. 7. II. 12.

Soil treatment	Seeds germinated after			Total germinating
	42 hrs.	47 hrs.	66 hrs.	
Untreated .....	62	223	464	480
Heated to 100° C. ....	63	224	468	486
" " 55° C. ....	101	269	468	479
Distilled water .....	97	263	470	484

Exp. 135, 67.

in comparison with distilled water, in the third case, however, some acceleration was produced by the extracts of untreated soil.

The experiment with the extract of the garden soil (B, Table V) was carried out immediately after that with the soil itself (C, Table III), but no resemblance can be discovered in the results. The conditions, however, are so very different that no strict comparison can be made. Soil behaves entirely differently from sand towards water and dissolved substances. The soil contained 37 per cent. of water and was not very wet, but the sand was absolutely flooded by the same amount so that we had to reduce the percentage to 19. There is no quantitative means of ascertaining when soil and sand are equally moist to seeds or plants, and any attempt to judge of equality in this direction reduces itself to mere guess work. A further difference between soil and sand is that soil has much the greater power of withdrawing dissolved substances from their solutions.

*The behaviour of the separate constituents.* Free ammonia has a remarkably powerful effect on germination, accelerating it at low concentrations (one or two parts per million), but retarding it at higher concentrations; at some intermediate point it has no effect (Table VI). The effective concentration is, however, influenced considerably by the amount of water present and the temperature. Thus when 19.3 per cent. of water was present four parts per million of ammonia caused a marked acceleration: when 20.6 was present the same amount of ammonia caused no acceleration but a slight initial retardation. A similar result was obtained with soil (Table IV) and we may therefore conclude that it is not due to any soil property. The determining factor in the case of ammonia appears to be the rapidity at which germination takes place; when germination is slow ammonia tends to hasten it, when it is rapid ammonia has less effect but tends to retard it. This is also seen in A and B of Table VI; two parts per million of ammonia considerably hasten germination in the slowly germinating seeds of B, but cease to do so in the more rapidly germinating seeds of A.

In Table VI, D a comparison is instituted between sodium nitrate, ammonium sulphate and free ammonia at equimolar concentrations, 180 parts per million being chosen to correspond with one of the soils under investigation. It will be seen that free ammonia is much the most drastic in its effect, ammonium sulphate comes next and sodium nitrate is the weakest. Further, the mixture corresponding with that in the soil behaves not unlike the soil itself, although, as already stated, no definite comparison can be made.

TABLE VI. *Effect of solutions of ammonia, ammonium sulphate and sodium nitrate on germination.*

A. Ammonia solutions added to pure sand. Turnip seed. 23. V. 11.

Moisture = 16.7 %.

Concentration parts of N. per million of moist sand	Seeds germinated in				Total germinating
	13 hrs.	18 hrs.	23 hrs.	61 hrs.	
Nil (water only) .	219	422	495	555	573
1 per million ...	248	453	495	559	575
2    "    .....	216	432	496	556	579
10   "    ... ..	117	389	483	559	576
100   "    ... .	none	none	none	none	none

Exp. 115, 61.

B. Ammonia solutions added to pure sand. Turnip seed. 26. VI. 11.

Moisture = 19.3 %.

Concentration parts of N. per million of moist sand	Seeds germinated in						Total germinating
	15 hrs.	18 hrs.	21 hrs.	24 hrs.	27 hrs.	36 hrs.	
Nil (water only).	48	224	368	459	551	572	600
1 per million .	67	255	394	460	553	575	592
2    "    .....	80	274	405	465	556	578	600
4    "    .....	97	278	423	465	551	579	604

Exp. 115, 74.

C. Ammonia solutions added to pure sand. Turnip seed. 3. VII. 11.

Concentration	Seeds germinated in							Total germinating
	16 hrs.	19 hrs.	22 hrs.	25 hrs.	28 hrs.	40 hrs.	64 hrs.	
19.3 % water, no NH <sub>3</sub>	38	194	352	423	465	553	586	598
4 per million ....	73	229	403	464	488	551	584	590
20.6 % water, no NH <sub>3</sub>	153	330	430	466	494	539	569	580
4 per million .....	139	337	427	459	485	539	576	587

Exp. 115, 77.

TABLE VI (cont.).

D. Comparison of  $(\text{NH}_4)_2\text{SO}_4$  and  $\text{NaNO}_3$  made up in concentrations equivalent to those present in a soil. (A 3 in Table III, 9. I. 12.)

The solutions added to pure sand. Turnip seed. 20. I. 12.

Moisture = 20 %.

Concentration parts of N. per million of moist sand	Substance added	Seeds germinated in				Total germin- ating
		20½ hrs.	23½ hrs.	40 hrs.	64 hrs.	
Nil (water only)	—	217	347	504	539	554
180	$\text{NaNO}_3$	125	264	515	569	575
180	$(\text{NH}_4)_2\text{SO}_4$	8	43	422	523	558
180	$\text{NH}_4\text{OH}$	0	0	0	41 <sup>1</sup>	165
90 as $\text{NH}_3$	$(\text{NH}_4)_2\text{SO}_4$	42	139	438	499	518
83 as nitrate	$\text{NaNO}_3$	—	—	—	—	—

Exp. 2, 61.

<sup>1</sup> By this time some of the  $\text{NH}_3$  had volatilised.

E. Sand.  $\text{NaNO}_3$  dilute solutions. Tomato seed.

Concentration	Seeds germinated after	
	40 hrs.	15 hrs.
14 parts N. per million	191	348
7 " "	222	365
1.4 " "	270	363
Distilled water	251	364

16. II. 12. Exp. 135, 70.

Tomato seed proved highly susceptible to free ammonia but was much less affected by solutions of sodium nitrate; it showed signs of being stimulated in germination by dilute solutions but retarded by stronger ones (E, Table VI).

*The subsequent growth of plants in partially sterilised soils.*

### 1. The seedling and young plant.

The general nature of the effects observed during the seedling and early stages have already been described. It now remains to give some of the details of the experiments.

*a. Soils heated to 100° C. Poor soils.* In poor soils there is almost invariably an acceleration in growth right from the outset. The food supply being the factor that limits plant growth, the increase in food supply consequent on partial sterilisation is accompanied by an increase

in growth. But another factor also comes into play. These poor soils are deficient in organic matter and therefore tend to be rather sticky, but after they have been heated their mechanical condition is often much improved. The young seedling therefore has a better chance of pushing ahead. On Feb. 24th tomato seeds were sown in poor arable soil ("Little Hoos," see Table II, A for composition); out of twenty sown in each set the numbers coming up were:

	Untreated soil	Soil heated to 100° C.	Soil heated to 55° C.	Soil treated with Toluene	Soil treated with CS <sub>2</sub>
March 10	1	5	1	2	1
" 11	3	8	6	4	4
" 13	7	11	8	9	5
" 14	7	14	8	9	5

and the spread of the cotyledons from tip to tip was:

" 13	1	7	2	3	1 cms.
" 14	2	11	4	4	1 "

In a warmer house the soils heated to 100° did not stand out so remarkably, but were no better than the soils treated with the toluene and carbon disulphide.

*Richer soils.* In richer soils a very different effect is produced. The seedlings are distinctly retarded in coming up. On March 6th 100 tomato seeds were sown in a glass house soil containing 0.33 per cent. nitrogen and losing 7.9 per cent. on ignition; the following numbers of seedlings appeared on the given dates:

	Untreated soil	Soil heated to 100° C.	Soil heated to 55° C.
March 25	10	0	5
" 26	14	1	12
" 27	18	3	17
" 28	24	6	21

and so on right through to the time when all were up.

This appears to be a new retardation additional to that affecting germination, for it continues to operate over many days, and is not confined to the germination period.

Poor seed suffers considerably in the soil heated to 100° and much of it does not yield plants at all. This causes a further marked



difference between the untreated and the steamed soils. Good seed, however, gives rise to the same number of plants in both cases.

Later on the roots begin to grow more quickly in the heated soil, even while the shoots are still retarded. This may happen during the cotyledon stage if the retardation has only been slight:

Soil MT, containing 0.26 % N, 1.0 %  $\text{CaCO}_3$  and losing 7.8 % on ignition.

25 tomato seeds sown Dec. 5th.

	Untreated soil	Soil heated to 98° C.	Soil heated to 55° C.	Soil treated with Toluene	Soil treated with carbon disulphide
Number up on Dec. 19	4	2	8	4	6
" " 20	4	4	10	6	6
" " Jan. 9	5	8	15	10	10
Spread of cotyledons on Jan. 18	3.6	3.3	4.1	2.5	2.5 cms.
Root length on Jan. 18	0.9	2.7	2.7	3.4	2.6 "

or it may be later on when the fourth leaves are out that quicker growth starts. Retardation at this later stage is shown in Fig. 5. Plants lifted at this early stage were found to have the following average weights per plant:

	Untreated soil	Soil heated to 98° C.	Soil heated to 55° C.	Soil treated with Toluene	Soil treated with carbon disulphide
Shoot, green weight grms.	0.65	0.31	1.33	1.27	1.06
" dry " "	0.07	0.03	0.13	0.115	0.085
Root, fresh " "	0.08	0.03	0.20	0.18	0.10
" dry " "	0.007	0.004	0.020	0.018	0.009

*b. Soils heated to 55° C. or treated with toluene and carbon disulphide.*

These generally cause an acceleration (especially the soil heated to 55°) but sometimes a retardation. Tomatoes sown on Feb. 24th in a greenhouse compost containing 0.35 % N, 0.11 %  $\text{CaCO}_3$  and losing 9.0 % on ignition came up as follows:

	Untreated soil	Soil heated to 100° C.	Soil heated to 55° C.	Soil treated with Toluene
March 8	3	0	2	4
" 9	6	8	5	10
" 10	9	9	9	13
" 11	12	10	15	15
" 13	15	15	17	17
" 14	17	16	17	18

20 seeds were sown in each set.

The spread of the cotyledons in cms. was :

		Untreated soil	Soil heated to 100° C.	Soil heated to 55° C.	Soil treated with Toluene
March	9	3	0	1	4
„	10	5	4	3	8
„	11	9	9	9	13
„	13	13	11	14	17
„	14	15	13	17	17

The experiment was made in duplicate in another house at rather higher temperature but with entirely different results, the plants on the treated soils being all behind those on the untreated soil. Subsequent repetition in the same house gave a result similar to the one set out above, treatment of the soil with toluene and carbon disulphide causing a slight acceleration while heat at 100° C. caused a marked retardation. This variation in the results is typical, and illustrates the close relationship between acceleration and retardation in these early stages of plant growth.

## 2. *The adult plant.*

*The times of flowering and ripening.* Tomatoes, cucumbers and chrysanthemums growing on the partially sterilised soil flowered several days before those on the untreated soil, and the fruit of the first two was not only earlier but more prolific. This fact is remarkable because as a general rule excess of nitrogenous food (such as may be supposed to result from partial sterilisation) retards flowering and ripening; the tomato grower greatly objects to rank growth as being detrimental to fruit production. Fig. 1 (p. 254) shows the total weight of fruit obtained from tomato plants grown on untreated and partially sterilised soils, the manuring and all other conditions being uniform for the whole series. The plants were grown in pots and stopped at four trusses. Details of the experiment are recorded in the *Journal of the Board of Agriculture*, 1913, **19**, 809, and need not be repeated here.

Fig. 7 shows blooms of chrysanthemums grown under similar conditions; the untreated soil is seen to give poorer growth and later blooms than the treated soils excepting only the steamed soil. With other varieties the plants on the steamed soil were ahead of those on the untreated soil; the blooms were also larger and brighter.

To some extent our result is due to the conditions of the experiment; the bad effect of rankness is much less evident when the plants are

grown in pots than when they have the less restricted root range of a border.

*Composition of the dry matter.* Various tables of analyses are given in earlier publications showing the composition of the dry matter of crops grown under similar conditions on partially sterilised and on untreated soils<sup>1</sup>. The percentage of nitrogen in the dry matter is in almost all cases found to be higher on crops grown on partially sterilised soils as also is the percentage of phosphoric acid where the soil has in the past been well dunged (*e.g.* the hop garden soil in the 1907 paper).

In further confirmation of these results the following may be given:

*Percentage of nitrogen in dry matter of barley, 1910 crop.*

Soil untreated	2.83	Soil untreated	2.98	Soil untreated	3.43
„ treated with		„ heated to 45° C.	3.31	„ dried at 40° C. for 24 h s.	3.39
Toluene	3.44	„ „ 55° C.	3.61	„ „ „ 5 days	3.56
CaS	3.32	„ „ 65° C.	3.67	„ „ „ 10 „	3.62
Shell petrol	3.39	„ „ 75° C.	3.55		
Ether	3.33	„ „ 85° C.	3.43		
		„ „ 100° C.	3.58		

Subsequent crops on the same soil gave similar results. The exception to the general rule occurs when the plants on the partially sterilised soils have made conspicuously more growth than those on the untreated soil. For example tomato plants pulled up just before flowering time after a period of markedly accelerated growth on the partially sterilised soils gave the following results:

Soil treatment	Untreated	Heated to 98° C.	Treated with Form-aldehyde	Treated with Pyridene	CaS	Petrol	Toluene	Phenol
Dry matter grams	7.1	26.8	16.6	13.2	13.2	12.1	11.8	9.0
N. % in dry matter	2.92	2.52	2.22	3.17	2.67	2.46	2.50	2.43

Analyses have been made of tomato plants left to grow till fruiting had finished so as to ascertain how far the nitrogen, phosphorus and potassium had been exhausted from the roots and stems, etc. and transferred to the fruit. The process was found to take place more completely on partially sterilised than on untreated soils. The fruit of plants raised on partially sterilised soil contained in most cases a higher percentage of nitrogen, and if the soil had previously been

<sup>1</sup> This *Journal*, 1907, 2, 313 *et seq.*; 1909, 3, 122.

TABLE VII. *Composition of dry matter of tomato plants grown in untreated and in partially sterilised soils.*

1. Amounts of Nitrogen, per cent. of dry matter and weight in grams per plant.

Pasture Soil 7F containing 0.59 % N, 0.15 % CaCO<sub>3</sub> and losing 13.5 % on ignition. This soil had not received dung.

	Weight per plant of dry matter in			Nitrogen per cent. in dry matter of			Weight of Nitrogen in			Total weight of	
	Root	Stem and leaf	Fruit	Root	Stem and leaf	Fruit	Root	Stem and leaf	Fruit	Dry matter	Nitrogen
Untreated	4.94	19.15	4.70	2.05	1.13	2.91	0.101	0.216	0.137	28.79	0.45
Heated to 98° C.	2.77	25.45	18.56	1.58	1.47	3.04	0.044	0.373	0.565	46.78	0.98
" + Basic slag	1.73	22.89	19.45	1.91	1.55	3.11	0.033	0.355	0.606	44.07	0.99
" 55° C.	2.99	22.66	17.68	1.61	1.16	2.06	0.048	0.261	0.364	43.33	0.67
Treated with Toluene	2.57	21.69	18.94	1.81	1.08	3.01	0.047	0.234	0.569	43.20	0.85
" " CS <sub>2</sub>	2.34	22.15	16.81	1.58	1.17	3.06	0.037	0.259	0.514	41.30	0.81

	Weight per plant of dry matter in			Nitrogen per cent. in dry matter of			Weight of Nitrogen in			Total weight of	
	Root	Stem and leaf	Fruit	Root	Stem and leaf	Fruit	Root	Stem and leaf	Fruit	Dry matter	Nitrogen
Untreated	5.14	16.23	9.03	2.30	1.01	1.72	0.118	0.163	0.156	30.39	0.44
Heated to 98° C.	6.84	31.25	24.40	1.57	0.82	2.12	0.107	0.255	0.516	62.49	0.87
" + Basic slag	9.97	29.52	28.40	1.39	0.86	1.94	0.138	0.254	0.551	67.89	0.94
" 45° C.	4.06	18.31	14.88	2.36	1.13	1.83	0.096	0.206	0.272	37.24	0.57
Treated with Toluene	4.05	23.76	16.74	2.35	1.04	2.00	0.095	0.248	0.334	44.55	0.68
" " CS <sub>2</sub>	3.95	22.78	17.56	2.41	1.01	1.73	0.095	0.229	0.304	44.29	0.63

Soil MC. Old tomato-house soil.



Soil *WB*. Old cucumber-house soil containing 0.72 % N, 0.92 %  $\text{CaCO}_3$  and losing 19.9 % on ignition.

	Weight per plant of dry matter in			Nitrogen per cent. in dry matter of			Weight of Nitrogen in			Total weight of	
	Root	Stem and leaf	Fruit	Root	Stem and leaf	Fruit	Root	Stem and leaf	Fruit	Dry matter	Nitrogen
Untreated .....	2.10	23.67	13.87	1.99	1.25	2.22	0.042	0.296	0.307	39.64	0.65
Heated to 98° C. ....	8.53	38.64	25.77	1.60	1.17	2.22	0.136	0.453	0.571	72.94	1.16
" + Basic slag .....	2.28	26.60	26.60	1.57	1.24	2.65	0.130	0.494	0.704	74.75	1.33
" 55° C. ....	2.03	29.13	11.70	2.09	1.10	2.08	0.042	0.320	0.243	42.86	0.61
Treated with Toluene .....	2.49	32.87	13.85	2.17	1.06	2.18	0.054	0.348	0.301	49.21	0.70
" " $\text{CS}_2$ .....	1.99	26.74	15.75	2.29	1.24	2.42	0.045	0.332	0.381	44.48	0.76

2. Amounts of phosphorus (expressed as  $\text{P}_2\text{O}_5$ ), per cent. of dry matter and weight per plant. Estimated as  $\text{P}_2\text{O}_5$  in ash.  
Soil *TF* containing 0.16 %  $\text{P}_2\text{O}_5$  soluble in boiling conc.  $\text{HCl}$  and 0.016 % soluble in 1 % Citric acid.

Per cent. of N, etc. given above.

	Weight per plant of dry matter in			$\text{P}_2\text{O}_5$ per cent. in dry matter of			Weight of $\text{P}_2\text{O}_5$ in			Total weight of	
	Root	Stem and leaf	Fruit	Root	Stem and leaf	Fruit	Root	Stem and leaf	Fruit	Dry matter	$\text{P}_2\text{O}_5$
Untreated .....	4.94	19.15	4.70	0.560	0.590	1.458	0.028	0.113	0.069	28.79	0.21
Heated to 98° C. ....	2.77	25.45	18.56	0.340	0.257	—	0.009	0.065	—	46.78	—
" + Basic slag .....	1.73	22.89	19.45	0.311	0.299	0.986	0.005	0.068	0.192	44.07	0.27
" 55° C. ....	2.99	22.66	17.68	0.356	0.361	0.853	0.011	0.082	0.151	43.33	0.24
Treated with Toluene .....	2.57	21.69	18.94	0.565	0.186	1.103	0.015	0.040	0.209	43.20	0.26
" " $\text{CS}_2$ .....	2.34	22.15	16.81	0.333	0.319	1.379	0.008	0.071	0.232	41.30	0.31

TABLE VII.—*continued*.Soil *MT* containing 0.39 %  $P_2O_5$  soluble in boiling conc. HCl and 0.23 % soluble in 1 % Citric acid.

	Weight per plant of dry matter in			$P_2O_5$ per cent. in dry matter of			Weight of $P_2O_5$ in			Total weight of	
	Root	Stem and leaf	Fruit	Root	Stem and leaf	Fruit	Root	Stem and leaf	Fruit	Dry matter	$P_2O_5$
Untreated .....	9.53	32.12	25.23	0.023	0.375	0.854	0.059	0.120	0.215	66.88	0.39
Heated to 98° C. ....	8.73	55.58	39.19	0.564	0.547	1.084	0.049	0.304	0.425	103.50	0.78
" 55° C. ....	5.03	33.52	26.23	0.596	0.400	1.070	0.030	0.134	0.261	64.78	0.44
Treated with Toluene ..	8.10	35.50	33.65	0.699	0.291	0.967	0.057	0.103	0.325	77.26	0.49
" " $CS_2$ ....	8.37	33.99	34.29	0.610	0.348	1.020	0.051	0.118	0.350	76.65	0.52

3. Amounts of potassium (expressed as  $K_2O$ ), per cent. of dry matter and weight per plant.Soil *TF* containing 0.39 %  $K_2O$  soluble in boiling conc. HCl and 0.08 % soluble in 1 % Citric acid.

	Weight per plant of dry matter in			$K_2O$ per cent. in dry matter of			Weight of $K_2O$ in			Total weight of	
	Root	Stem and leaf	Fruit	Root	Stem and leaf	Fruit	Root	Stem and leaf	Fruit	Dry matter	$K_2O$
Untreated .....	4.94	19.15	4.70	1.720	2.519	6.149	0.085	0.482	0.289	28.79	0.86
Heated to 98° C. ....	2.77	25.45	18.56	0.919	1.318	—	0.025	0.335	—	46.78	—
" + Basic slag	1.73	22.89	19.45	0.567	1.626	5.487	0.010	0.372	1.067	44.07	1.45
" 55° C. ....	2.99	22.66	17.68	0.824	1.568	4.971	0.025	0.355	0.879	43.33	1.26
Treated with toluene ....	2.57	21.69	18.94	1.287	1.309	5.741	0.033	0.264	1.087	43.20	1.40
" " $CS_2$ ....	2.34	22.15	16.81	0.839	1.688	6.910	0.020	0.374	1.162	41.30	1.56

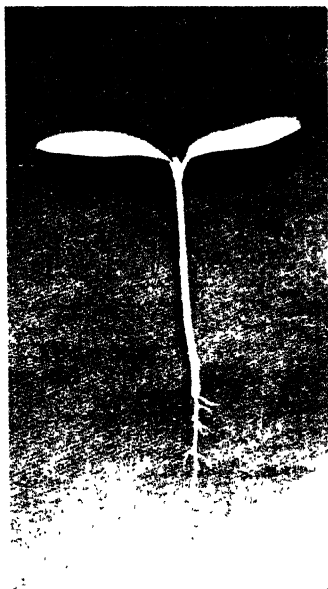




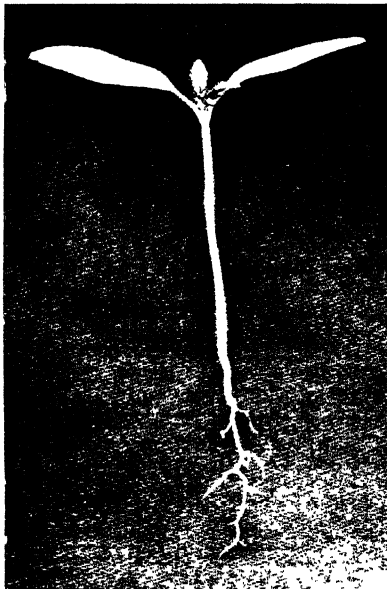
TABLE VII.—*continued.*

Soil MT.

	Weight per plant of dry matter in			CaO per cent. in dry matter of			Weight of CaO in			Total weight of	
	Root	Stem and leaf	Fruit	Root	Stem and leaf	Fruit	Root	Stem and leaf	Fruit	Dry matter	CaO
Untreated .....	9.53	32.12	25.23	0.242	7.074	0.065	0.023	2.272	0.016	66.88	2.31
Heated to 98° C. ....	8.78	55.58	39.19	0.374	6.346	0.026	0.033	3.527	0.010	103.50	3.57
" 55° C. ....	5.03	33.52	26.23	0.275	4.461	0.047	0.014	1.495	0.012	64.78	1.52
Treated with Toluene .....	8.10	35.50	33.66	0.231	6.908	0.038	0.018	2.452	0.013	77.26	2.48
" " CS <sub>2</sub> .....	8.37	33.99	34.29	0.298	5.797	0.051	0.025	1.971	0.017	76.65	2.01

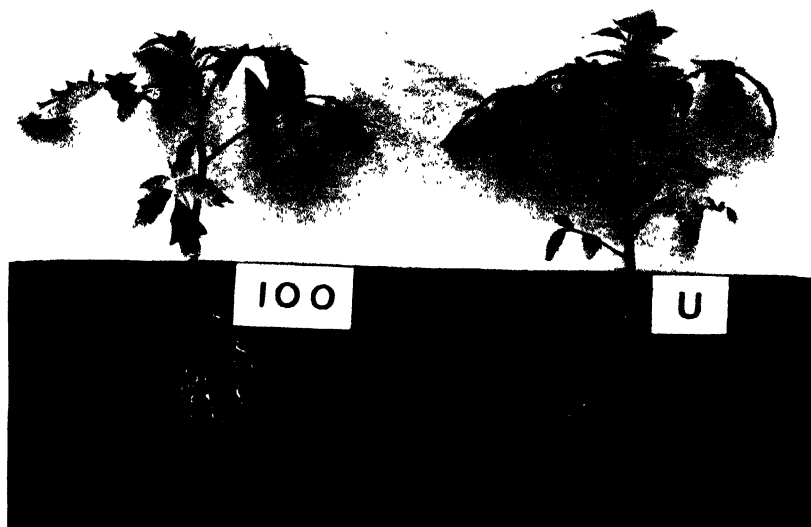


Soil heated to 100° C.



Untreated soil.

Fig. 2. Retardation in early stages of growth in soil heated to 100° C. Tomato seedlings.



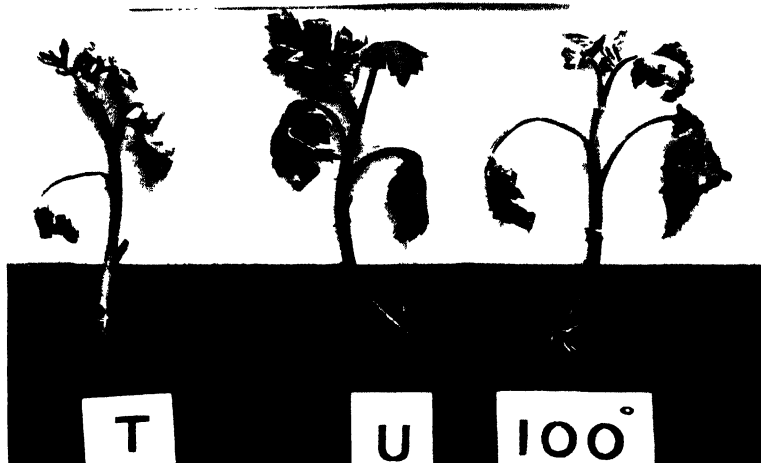
Soil heated to 100° C.

Untreated soil.

Fig. 3. Accelerated root development in later stages in soil heated to 100° C. Tomato seedlings.



## F. S. VALLIS



Soil treated with toluene.

Untreated soil.

Soil heated to 100 C.

Fig. 4a. Retardation in root formation in treated soils. Chrysanthemum cuttings, var. F. S. Vallis.

## REG. VALLIS

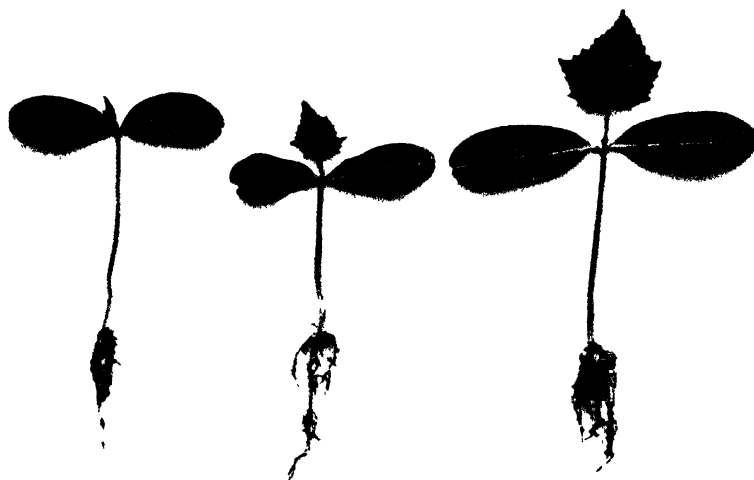


Soil heated to 100 C.

Untreated soil.

Fig. 4b. Accelerated root development in later stages in soil heated to 100° C. Chrysanthemum cuttings, var. Reginald Vallis.



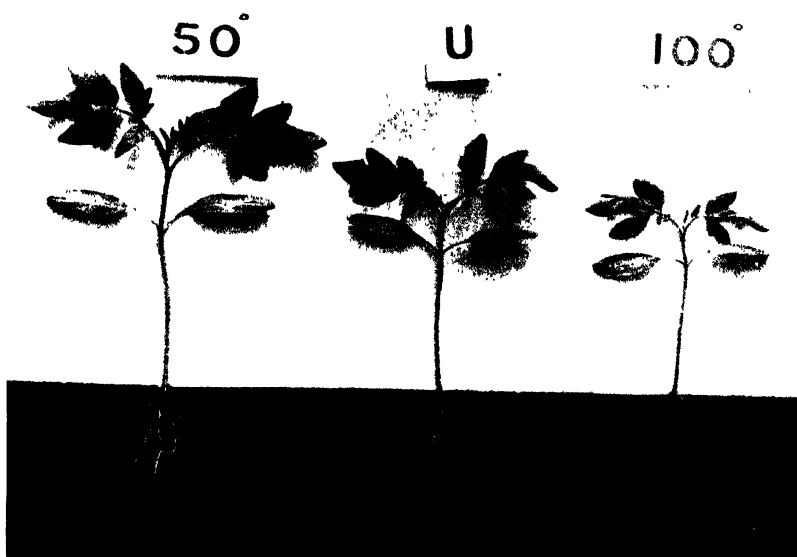


Soil heated to 100° C.

Untreated soil.

Soil heated to 55° C.

Fig. 5 a. Acceleration in early stages of growth in soil heated to 55° C. and retardation in soil heated to 100° C. Cucumber seedlings.



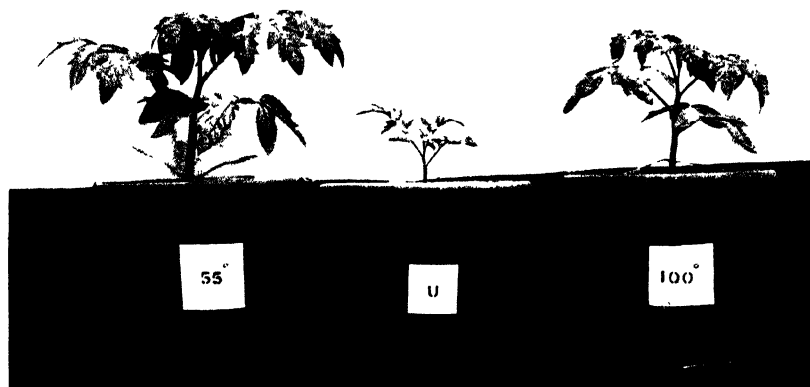
Soil heated to 50° C.

Untreated soil.

Soil heated to 100° C.

Fig. 5 b. Acceleration in early stages of growth in soil heated to 50° C. and retardation in soil heated to 100° C. Tomato seedlings.





Soil heated to 55° C.

Untreated soil.

Soil heated to 100° C.

Fig. 6. Remarkable acceleration sometimes produced later on in soil heated to 55° C. Tomato plants.

Soil treated with  
toluene.

Untreated soil.

Soil heated to  
100° C.Soil treated with  
calcium sulphide.

Fig. 7. Earlier flowering on some of the partially sterilised soils. Chrysanthemums, var. David Ingamells.





dunged, a higher percentage of phosphoric acid and of potassium than that of plants raised on untreated soil; it also had a sweeter taste. The roots of plants grown on soil heated to 100° contained less nitrogen, phosphoric acid and potash than those of plants grown on untreated soil. The roots of plants grown on soil heated to 55°, or treated with antiseptics, do not always show this relationship nor do the stems and leaves.

## SILVER-LEAF DISEASE (II).

By F. T. BROOKS, M.A.

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### INTRODUCTION.

THE present paper is an account of observations and experiments in connexion with Silver-leaf Disease which have been made since the publication of my paper on the same subject in the *Journal of Agricultural Science* for 1911. I am indebted to the Board of Agriculture for giving a grant to assist this work and to the Cambridge University School of Agriculture for placing at my disposal a plot of land on its farm for most of the fruit trees used in the experiments. I have also to thank Mr R. I. Lynch, Curator of the Botanic Gardens, Cambridge, for providing accommodation for other trees.

In a previous paper<sup>(1)</sup> on this disease further evidence was given of the ability of the fungus, *Stereum purpureum*, to induce the phenomenon of Silver-leaf and upon it the chief responsibility was laid for the losses caused by the malady in the fruit-growing districts of this country. In that paper (p. 141) I pointed out it would be rash to say that *Stereum purpureum* was the *only* cause of Silver-leaf. Recent investigations have strengthened this view, for specimens of silvered foliage have been seen which I am unable to attribute to the action of *Stereum purpureum*.

Subsequent remarks may be anticipated by saying that the silvering of foliage is a widespread phenomenon which is probably induced by various means, the chief one of which in the fruit-growing districts of this country being the fungus *Stereum purpureum*.

Since my paper was published in 1911, Mr H. T. Güssow<sup>(2)</sup> has written an account of the disease as it occurs in Canada. He also emphasises the part which *Stereum purpureum* plays in causing Silver-leaf disease in fruit trees, and in general his observations are in close agreement with my own.

All the silvered leaves I have examined exhibit the following features:—The upper epidermis is more or less loose from the palisade cells. There is a marked tendency for the mesophyll cells to fall asunder when sections of the leaf are cut, and so pronounced is this, that it is sometimes impossible to obtain sections of silvered leaves which will hold together. Cavities in walls of epidermal cells as described by Percival<sup>(8)</sup> are sometimes present but I agree with Güssow that they are not invariably found in silvered leaves. The phenomenon of silvering is primarily due to the accumulation of air either below the epidermal cells or in the cavities of their walls, the presence of air in these places interfering with the normal reflection of light from the surface of the leaf.

#### FIELD OBSERVATIONS.

The following supplementary account of the distribution of Silver-leaf should be read in connection with my earlier paper. As indicated there, it has been an almost constant experience to find fructifications of *Stereum purpureum* growing on dead branches of Plum trees which bear silvered foliage on other, living, branches. A photograph of such a tree is shewn in Fig. 1, Plate XII. As the death of such trees proceeds, *Stereum purpureum* develops in increasing abundance both on the branches and on the trunk. On one occasion the fructifications of *Polyporus adustus* as well as of *Stereum purpureum* were found to be growing from the trunk of a silvered Plum tree and *Fomes igniarius* has been seen growing in company with *Stereum purpureum* on several silvered Plum trees, but there is as yet no reason for thinking that either *Polyporus adustus* or *Fomes igniarius* is a cause of Silver-leaf. The latter fungus is frequently found on the branches and trunks of Plum trees which bear normal foliage.

In addition to "Victorias" and "Czars," trees of the following varieties of Plums have been seen to be affected with Silver-leaf: Early Rivers, Pond's Seedling, Monarch, Gisborne, Transparent Gage, Purple Gage, and Green Gage, but these varieties are much less susceptible to the disease than are "Victorias" and "Czars." A large number of branches of silvered Plum trees belonging to various varieties and growing in many different fruit gardens have been examined in order to ascertain whether discoloured wood was constantly present in them or in other portions of the tree with which these branches were directly connected. Such discoloured wood has been

found to be invariably associated with the silvered foliage of Plum trees gathered from plantations and this discolouration (cf. Plate XIII, Fig. 4) is strictly comparable with that found in branches artificially inoculated with *Stereum purpureum*. Stained longitudinal sections of such wood shew abundant hyphae in the water-conducting elements which are often partly filled with a gummy substance to which the discolouration is primarily due. The diseased wood is frequently found a considerable distance below the silvered foliage. The amount of discoloured wood as seen in transverse sections varies considerably, a sector of less than  $90^\circ$  being sometimes present, while at other times an area of more than  $180^\circ$  is dark brown in colour. In these cases the zone of discoloured wood sometimes extends from pith to cambium but more often, as in Fig. 4, a narrow band of healthy wood remains in contact with the cambium. Occasionally several discoloured zones are scattered amongst the healthy tissues. Longitudinally the discoloured wood extends a considerable distance and it can frequently be traced back to the larger branches and to the trunk of the tree. The bark of a diseased branch is not affected as soon as the wood, but once it is attacked, the fungus advances in it, as in the wood, more rapidly in a longitudinal than in a lateral direction. Trunks of Plum trees which have been cut down in an advanced stage of disease always contain a large amount of discoloured wood. Fructifications of *Stereum purpureum* frequently develop on the dead bark of one side of a branch or trunk even though the opposite side is still sound.

The distribution of silvered trees in a plantation of Plum trees is usually sporadic unless the plantation is badly neglected, but in a number of cases in which Plum trees have been cut back for various reasons Silver-leaf has developed on almost every tree subjected to this operation. In one such case the larger branches of twenty healthy Plum trees about 20 years of age were cut back during the autumn of 1910 so that only 2 or 3 feet of each of these branches remained. Branches put forth during 1911 were re-grafted in the spring of 1912. None of these grafts grew and when the trees were examined in May 1912 side shoots arising from the branches and trunk and also suckers springing from the root stock were seen to be silvered. Each one of the twenty trees was affected in the same manner. During October, fructifications of *Stereum purpureum* developed in enormous numbers on each of these trees, both on the branches and on the trunk. The large surfaces exposed when the trees were cut back had not been protected in any way and one can readily imagine the facility given to

*Stereum purpureum* to effect an entrance by this neglect. The fact that fructifications developed at the base of the trunks of certain trees during October 1912 shewed that the fungus had made rapid progress in the tissues.

During the early part of 1912, Mr W. O. Backhouse, late of the John Innes Horticultural Institution, and Mr M. A. Bailey of the same Institution informed me that some seedling Plums which were growing there exhibited the phenomenon of Silver-leaf. Mr Backhouse gave me the following particulars about these seedlings. The seeds were germinated in 2 inches of soil in boxes and the seedlings became silvery at about the time the roots became cramped. When the seedlings were potted they began to recover their normal appearance. The percentage of seedlings which became silvered before being potted varied in different varieties. Thus "Victorias" were affected to an extent of about 50 per cent., "Magnum Bonums" to about 20 per cent., and "Pershores" also to some extent. It is noteworthy that "Czars" grown under the same conditions were entirely unaffected although this variety in fruit plantations is frequently attacked by Silver-leaf disease. The seeds from which these seedlings were derived were obtained from healthy trees. The "Victoria" seedlings which came from "selfed" flowers were more affected with Silver-leaf than those derived from flowers "naturally" pollinated. Some of these seedlings were sent to me and I examined them with the following results: The silvering was strictly comparable with that which occurs in adult trees. The epidermis was partly free from the underlying palisade tissue and on trying to cut sections of the leaf there was a decided tendency for the mesophyll cells to fall asunder one from the other. There was no evidence of fungus attack in either leaf, stem, or root. As already stated, the seedlings began to recover when given more room in which to grow and upon examining them in August, I saw that the recovery of the foliage to its normal appearance was well advanced. I came to the conclusion that in such a case as this the phenomenon of Silver-leaf was not caused by *Stereum purpureum*. Güssow<sup>(b)</sup> mentions some cases of the appearance of Silver-leaf in seedling plums, and suggests the possibility either that the disease may be perpetuated by means of the seed or that the seedlings may become infected at a very early stage.

For two years in succession Silver-leaf has been reported on Sloe trees (*Prunus spinosa*) in the Isle of Wight. By the kindness of Miss E. Dale I have been able to examine foliage of one of these trees and

the silvering has been found to be strictly comparable with that occurring in cultivated Plum trees.

Although I have seen comparatively few adult Apple trees shewing Silver-leaf I have frequently found scions of regrafted trees that are silvered.

One group of regrafted Apple trees ("Blenheim Orange") which I saw last summer may be described. Fifteen of these trees were cut back three years ago and were regrafted with scions of either the "Grenadier" or "Jubilee" variety. The grafts grow until the spring of 1912 when those on twelve of the trees rapidly died. When these trees were examined in August, 1912, each stock shewed innumerable sporophores of *Stereum purpureum* growing from the bark, and there can be little doubt that this fungus killed the trees (Plate XIII, Fig. 5). The top of one of these trees was sawn off and examined in detail in the laboratory. When split longitudinally the upper part of the stock was seen to be almost completely discoloured, but there was more healthy wood in one of the grafts, thus shewing that it was the stock which had been most severely affected. Hyphae were present in abundance in sections of the discoloured wood. Some portions of the bark of the stock were still healthy and on these no fructifications were found. Grafts of the other three trees were living and were silvered. In longitudinal section the upper part of the stock shewed much less discoloured wood than in the case previously described and the graft was quite sound. Hyphae were found in sections of the discoloured wood of the stock. Such a tree was probably attacked by *Stereum purpureum* but less severely than the trees bearing dead grafts.

Another example of failure of regrafted Apples may be described. In the spring of 1911 four Apple trees ("Cox's Orange Pippin") were regrafted. None of the grafts "took" and by the autumn of the same year *Stereum purpureum* was growing in great abundance on the exposed surfaces of the stock. One of these trees was subsequently cut back to within 9 inches of the ground and the exposed surface covered with clay. At a later date fructifications of *Stereum purpureum* developed even over the clay.

As already mentioned in my previous paper, silvering of Apple grafts is of frequent occurrence even when the scions grow vigorously. All the leaves of such grafts or only a few of them may become silvered. This happens frequently in Cambridgeshire when scions of "Bramley Seedling" are used, but in a large number of these cases the shoots grow out of the malady in the course of time. In one plantation

21 out of 29 Apple trees regrafted with scions of "Bramley Seedling" shewed silvering of the leaves during 1912. It is doubtful whether all such examples of Silver-leaf are due to *Stereum purpureum* for in some which have been examined the amount of discoloured wood in the upper part of the stock is small, the diseased tissues extending only an inch below the exposed surface three years after the stock was cut back. This amount of discolouration is no greater than frequently occurs during the processes of decay that normally affect the exposed extremities of trees which are not protected from the action of micro-organisms.

In a recent conversation Professor Barker of the National Fruit and Cider Institute told me he has seen Apple shoots which have been cut from healthy trees and kept in the air for a short time assume a silvery appearance. This affection which concerned "King of the Pippins" and other varieties of Apples appeared only during the autumn and was irregular in occurrence. I hope to have an opportunity of examining this phenomenon another year. Whether it is Silver-leaf or some other malady allied to it, the affection is evidently not due to the action of *Stereum purpureum*.

While writing of the genus *Pyrus* I may mention that a branch of a tree of *Pyrus prunifolia* (the Siberian Crab) growing at the Botanic Gardens, Cambridge, was silvered during the summer of 1912.

Several other silvered Gooseberry bushes and Red Currant bushes have been seen since the publication of my previous account; *Stereum purpureum* was growing on dead wood at the base of some of the Red Currant bushes.

A silvered Laburnum tree has been under observation during 1912. At the end of August last, fructifications of *Stereum purpureum* developed in abundance on a dead lateral branch. This tree had been severely pruned in previous years and the wounds thus made doubtless offered facility for the entrance of the fungus.

In February last I saw some plants of the White Dead Nettle (*Lamium album*) the leaves of which were silvered. This affection appeared to be the same in character as Silver-leaf in fruit trees. The upper epidermis was puckered and somewhat shrivelled in places and it could be readily torn away from the underlying mesophyll. It was difficult to obtain section of the silvered leaves on account of the tendency for the mesophyll cells to fall asunder. Other White Dead Nettle plants observed towards the end of March were seen to be silvered, but in this case only the older leaves of the shoots exhibited the phenomenon of silvering. No sign of fungus attack was visible in



these Dead Nettle plants, and the conclusion drawn was that in this case also Silver-leaf could not be attributed to *Stereum purpureum*.

#### THE HABITATS AND CHARACTERS OF *STEREUM PURPUREUM*.

Considerable attention has been paid to noting the various kinds of plants on which *Stereum purpureum* fructifies in nature. Sporophores of this fungus have been found in England on dead tissues (wood and bark) of the following trees: Plum, Apple, Red Currant, Willow (various species), Poplar (various species), Birch, Beech, Sycamore, and Laburnum, and doubtless this list will be extended with fuller observation. As is the case with many other fungi which also live a similar, partly saprophytic, partly parasitic, existence, fructifications of *Stereum purpureum* are developed only on dead tissues whether the fungus has been living recently as a saprophyte or as a parasite. As will be described later, pieces of sporophore obtained from a dead stump of a Birch tree in the midst of a wood readily induced Silver-leaf in Plum trees.

The closely allied fungus *Stereum hirsutum* is found commonly on dead Oak and Hazel but I have only once seen it on a member of the Plum family. In this case it was growing on a dead part of the trunk of a Damson, the foliage of which was healthy.

As is well known, the fructifications of *Stereum purpureum* are extremely variable in form (Plates XII and XIII, Figs. 1, 2, 3 and 5). Sometimes they are resupinate, and at other times profusely imbricate. On a branch of a Plum tree which is directly horizontally the fructifications are usually confined to the under surface, from which they project only a short distance. When the sporophores develop on the trunk or on a branch which is directed upwards they are usually densely imbricate. When young the sporophores are purplish in colour but they become dingy with age. The sterile surface is hairy when free from the substratum. The fructifications of *Stereum purpureum* may be found at all times of the year. They develop from the vegetative mycelium only after a spell of wet weather and appear in particular abundance after heavy rains during the autumn. The capacity of the sporophores to dry up and spore again under moist conditions has been referred to in the previous paper. According to my experience the spores are  $5-7\mu \times 3-4\mu$  in size, these dimensions being rather smaller than those given by Massee<sup>(6)</sup>.

A method of growing the fungus from spores on blocks of Plum wood has already been described<sup>(2, 3)</sup>. The sporophores referred to there

have constantly lacked the consistency and form of sporophores as they occur in nature. Nevertheless basidia and spores have been frequently observed on these rudimentary sporophores. Miss Wakefield<sup>(13)</sup> has pointed out the probability of the existence of physiological varieties of *Stereum purpureum* some of which produce sporophores in artificial culture while under the same conditions others give rise only to vegetative mycelium.

#### INOCULATION EXPERIMENTS.

The results of a considerable number of inoculation experiments have been already reported. Those given below refer chiefly to inoculations which have not been previously described.

##### (1) *Inoculation Experiments with Plum trees.*

Inoculations with moist pieces of fructifications of *Stereum purpureum* taken from silvered Plum trees have continued to cause Silver-leaf in Victoria and Czar trees almost invariably. One "Czar" inoculated in this manner during 1911 shewed Silver-leaf in the spring of that year but during the summer it became leafless. No leaves were put forth in the spring of 1912 and during the autumn fructifications of *Stereum purpureum* developed in profusion on the main stem, from the level of the soil upwards. It is evident that this tree was killed by *Stereum purpureum*. The only failure to be recorded in connexion with these inoculations of Victoria Plums concerns three young bushes, the main stems of which were inoculated during December 1911. The foliage of the spring of 1912 was normal and no signs of Silver-leaf developed during the summer. Gumming, however, occurred at the places of inoculation. Two other bushes inoculated at the same time and in the same way produced silvered foliage the following spring. It is difficult to account for these failures. It is possible that for some reason not understood the mycelium failed to establish itself in the tissues with sufficient vigour to cause Silver-leaf and the affection may yet appear next spring, for in some other experiments its appearance has been delayed more than a year. The vigour of the sporophores used in these and similar inoculations was always tested by ascertaining whether control pieces would deposit spores under suitable conditions.

As would be anticipated, the silvering exhibited in the spring is usually the more extensive the longer the inoculation has been made.

When an inoculation is made during the autumn the inoculated branch sometimes dies during the winter, so that no leaves are put forth from the buds of this branch; silvering is however shewn by leaves developed on neighbouring branches. Inoculations made after July frequently do not result in silvering of the foliage until the following spring, but one inoculation of a Victoria Plum made during August 1912 caused silvering less than five weeks later. The leaves which became silvered were fully formed before the inoculation was made.

Salmon<sup>(11)</sup> has suggested the possibility of the existence of different strains or varieties of *Stereum purpureum*, some causing Silver-leaf and others being inactive in this way. The previous experiments of Spencer Pickering<sup>(6, 10)</sup> and myself<sup>(8)</sup> shew that there is probably nothing in the nature of specialised parasitism in the biology of this fungus. The mode of life of a wound parasite differs widely from that of an obligate parasite such as a Mildew or a Rust fungus, and there is less likelihood of a particularly specialised mode of nutrition being evolved in the case of a fungus which may live either saprophytically or parasitically than in one which is an obligate parasite. *Stereum purpureum* is, however, such a common saprophytic fungus upon various kinds of woody tissues that it is a matter of importance to ascertain if the fungus taken from tissues where it is not associated with Silver-leaf can produce this phenomenon in Plum trees. The following experiments in this connexion have been performed. Small portions of the sporophores of the fungus obtained from a dead Birch stump in the midst of a wood were placed in 12 branches belonging to two different Victoria plum trees, 4—5 years of age, during October 1911. In April 1912 when some of the buds opened, all of the 12 inoculated branches shewed silvered leaves. Many of the foliage buds of the inoculated branches, however, did not open, and the leaves which did develop, rapidly died so that the inoculated branches became bare. Eight of the inoculated branches were leafless by the end of April and some shoots which had meanwhile developed on the main stem of one tree two and a half feet below the level of the inoculations had become silvered. By the middle of June all the inoculated branches were leafless and the leaves of shoots which were being thrown up by the root stock were silvered. In this connection it is to be noted that all the Plum trees which have become seriously affected with Silver-leaf after inoculation have put forth numerous shoots both from the base of the stem and also from the root stock as though the trees were making strenuous efforts to free themselves from the disease, Fig. 6 is a

photograph of one of these trees taken in the early part of October 1912. It will be noticed that the tree is bare except for the silvered shoots which have developed on one side of the base. At the end of the month fructifications of *Stereum purpureum* began to arise on one side of the trunk of one of these trees throughout its whole length and were especially well developed at soil level on the side opposite that on which the silvered suckers had been thrown up. The tissues of the main stem were dead only on one side and the fructifications arose only from this region. The moist condition of the soil probably accounts for the particularly strong development of fructifications at this level. During the following month *Stereum purpureum* developed in much the same way on the main stem of the other Plum tree inoculated with pieces of sporophore obtained from a Birch stump. It is thus clear that these trees are dying with great rapidity. The trees were perhaps somewhat more severely inoculated than other trees inoculated with *Stereum purpureum* obtained from a silvered Plum tree but, taking this into consideration, they are undoubtedly being more rapidly killed than the latter. These experiments show that *Stereum purpureum* from such a source as a dead Birch stump may be at least as virulent in causing Silver-leaf as *Stereum purpureum* obtained from a silvered fruit tree. Indeed these results engender a suspicion that the fungus may be all the more aggressive for a change of "host."

Additional inoculations with the mycelium of *Stereum purpureum* cultivated in pure culture from spores have been followed by silvering of the foliage in a high percentage of cases. Thus 12 inoculations made in three "Victorias" with this mycelium during December 1911 all resulted in Silver-leaf the following spring. On the other hand 6 inoculations of a "Monarch" and 6 inoculations of an "Early Rivers" made during March 1912 have not hitherto resulted in Silver-leaf. These varieties are not so susceptible to Silver-leaf as the "Victoria" and may possibly resist the disease in these experiments. Two examples of the results of inoculating "Victorias" with the cultivated mycelium will be described in detail.

In one, six branches of a young bush tree were inoculated with cultivated mycelium in December 1911. At the end of April 1912, five of the inoculated branches bore silvered foliage, the other inoculated branch being leafless and apparently dead. Five uninoculated branches also carried silvered leaves. Large masses of gum were extruding both from the places of inoculation and from other points, whereas in control trees of the same variety there was only a trace of gumming. By the

middle of August all the inoculated branches were leafless. On one of the uninoculated branches which shewed silvered leaves in April a "midsummer shoot" had developed but none of its leaves became silvered.

In the other case three branches of a young bush tree were similarly inoculated in December 1911. At the end of April 1912 all the branches, inoculated and uninoculated, shewed silvered leaves and there was profuse gumming at the points of inoculation and at places on the main stem. By the middle of August the general appearance of the bush was about the same, but in this instance some of the leaves at the proximal end of a "midsummer shoot" were silvered.

In the paper of 1911 it was pointed out that of 38 branches of "Czars" and "Victorias" inoculated with the cultivated mycelium 10 became affected with Silver-leaf during that year. During 1912 eight of these inoculated branches were silvered and three of them were branches which did not carry silvered leaves the previous year. Some branches affected during 1911 have evidently recovered.

It was recorded in the same paper that a small number of branches of old Victoria plums inoculated with spores of *Stereum purpureum* had been followed by Silver-leaf. These successful spore inoculations were made during the summer of 1910 and silvering of the foliage resulted in 1911. In these cases spores were inserted into a T-shaped wound immediately after the latter had been made. No such success has followed any of the spore inoculations made in the same way during 1911 in branches of young Victoria trees.

A few weeks ago, another of the branches inoculated with spores during the spring of 1911 but which remained unsilvered during 1912, was cut off in order to trace the progress made by the fungus from the place of inoculation. It was found that a zone of discoloured wood covering about 90 degrees extended from an inch above the wound to  $3\frac{1}{2}$  inches below it, the diseased tissues being entirely surrounded by healthy wood. A similar branch cut off 18 months before shewed a zone of discoloured wood extending from three quarters of an inch above to 2 inches below the place of inoculation. It is therefore evident that the fungus has not made rapid progress recently.

On the other hand certain spore inoculations made in older wounds of branches of similar trees have been followed by Silver-leaf. These experiments will be described in detail. They concern two trees, one a "Czar" and the other a "Victoria," each being a half-standard 4—5 years old. In March 1911 six branches of each tree were broken across

so that they were half severed. The wounds thus made were exposed to the action of the weather for three weeks after which two branches of the "Victoria" were cut off above the wounds. At the same time spores of *Stereum purpureum* were inserted in the wounds of the twelve branches. The wounds were then covered with tinfoil and wool and each of the ten partly severed branches was bound in such a manner as to prevent it from being broken off by the wind. None of the inoculated branches shewed Silver-leaf during the summer of 1911 but during the spring and summer of 1912 three inoculated branches of each tree, i.e. six inoculated branches in all, carried silvered foliage. In regard to the Czar tree, two branches bore silvered leaves both above and below the places of inoculation; on the other branch only leaves below the wound were affected. Subsequently these three branches together with another inoculated branch which bore only healthy leaves, broke off at the places of inoculation on account of the heavy crop of fruit. In the case of the "Victoria" only one of the inoculated branches showed silvered leaves in April 1912 but two other uninoculated branches closely connected with it also exhibited Silver-leaf. Leaves of another inoculated branch the upper part of which had broken off, became silvered in June and immediately below it an uninoculated branch also carried silvered leaves. Later in the summer leaves of a third inoculated branch, the upper part of which had broken off, became silvered. It may be said here that several branches of other trees kept as controls also became partly severed and hung down during the summer but none of the leaves of these branches shewed Silver-leaf. One of the larger branches of the "Victoria" that carried silvered leaves and to which one of the inoculated branches was attached, was brought into the laboratory during October in order to ascertain what effect the inoculation had had upon the tissues. Passing downwards from the place of inoculation nothing but dead tissue was found until a lateral shoot bearing silvered leaves was reached two inches below, where about half the wood and bark, as seen in cross section, was discoloured in the same way as has been previously described in connection with my other investigations of Silver-leaf disease. Two inches further below, discoloured wood extended over about a quarter of the cross section. Hyphae were present in abundance in longitudinal sections of this zone of diseased wood. The lateral shoot which bore silvered leaves contained no discoloured wood immediately above its junction with the inoculated branch, hence the silvering of its leaves is to be probably attributed to the fungus present

in the diseased wood below. For the following reason as well as for the fact that the wounds were exposed for three weeks before inoculation it is impossible to say with certainty if the mycelium present in the discoloured wood was derived from the spores inserted at the time of inoculation. This wood is in direct continuity with the dead wood immediately below the place of inoculation but, unfortunately, pruning wounds are present on the branch and, as explained below, these prevent one from drawing a more definite conclusion as to the influence of the inoculation. Below an old pruning wound a certain amount of discoloured wood is invariably found, the discolouration being due to the action of various micro-organisms; the dead wood does not usually extend far back but in some cases I have found it two inches below the exposed surface. Hence it is uncertain how much of the tissue has been killed by the influence of pruning wounds and how much by the mycelium derived from the spores used at the time of inoculation. Silver-leaf has not developed in connection with the presence of pruning wounds on any of the other 70 or more young Plum trees used in the investigation and this is a strong argument in favour of the interpretation that the occurrence of Silver-leaf described above, together with the five other cases associated with it, was probably caused by the inoculation of a previously wounded branch with spores of *Stereum purpureum*. This interpretation supports the view that the fungus behaves as a wound-parasite in fruit plantations. Another possible interpretation of these results is that Silver-leaf developed as a consequence of the manner in which the trees were mutilated apart from any influence due to the insertion of the spores. However, Silver-leaf did not appear until the spring of 1912 and had the phenomenon been caused by pathological conditions outside the category of parasitic influences one might have expected it to become manifest during 1911. It is known from previous experiments that the fungus inserted in the form of spores takes considerable time to advance in the tissues sufficiently to cause Silver-leaf, so it is not surprising that the affection did not appear until 1912.

It is of interest to note that none of the control inoculations performed last year with pieces of dead sporophores of *Stereum purpureum* and with pieces of living sporophores of *Stereum hirsutum* and *Polystictus hirsutus* have been followed by Silver-leaf. Inoculations made during the summer of 1912 with *Stereum rugosum*<sup>1</sup> have not yet

<sup>1</sup> I am indebted to Miss Wakefield, of the Herbarium, Royal Botanic Gardens, Kew, for kindly sending me material of *Stereum rugosum*.

resulted in silvering. Two branches of a Czar tree inoculated with *Stereum hirsutum* were investigated in the laboratory in order to see if the fungus had developed in the tissues. I found that it had made considerable progress in each branch. Thus in one branch there was a zone of discoloured wood which extended from four inches below the point of inoculation to two inches above it, the average amount of diseased wood within these limits being about one third of the area of the cross section. Longitudinal sections of the discoloured zone shewed the presence of hyphae. It is thus evident that *Stereum hirsutum* grows with some facility in the tissues of Plum trees but until the present the phenomenon of Silver-leaf has not been associated with this fungus. Gussow<sup>(6)</sup> has discovered that *Polystictus versicolor* and *Bjerkandera adusta* grow well in living tissues of Apple trees, but their development in them has not yet been followed by silvering of the foliage. Münch<sup>(7)</sup> also has pointed out that *Schizophyllum commune*, *Stereum rugosum*, *Stereum purpureum*, *Stereum hirsutum* and other fungi cause extensive discolouration of the sapwood of old trees when inserted by inoculation.

It should be noted that even in control experiments in which a scalpel wound is made in a branch and is subsequently covered with tinfoil and wool, a small amount of discoloured wood is subsequently found around the wound. This is doubtless due to the fact that in outdoor experiments of this kind it is impossible to carry them out under conditions which are completely sterile.

Recovery of silvered Plum trees has already been recorded in previous papers by Spencer Pickering<sup>(10)</sup> and myself<sup>(3)</sup>. One of the Czar trees which was conspicuously silvered in 1911 in consequence of inoculation with a portion of a sporophore of *Stereum purpureum* shewed only the merest trace of affection during 1912. Two branches belonging to different trees which exhibited Silver-leaf in 1911 after inoculation with cultivated mycelium of *Stereum purpureum* were entirely unaffected during 1912. In this connection mention should be made of the complete recovery during 1912 of a Laburnum which was silvered in 1911 in consequence of inoculation with *Stereum purpureum*. These cases of recovery may be considered to be due to a check in the growth of the fungus, possibly on account of active resistance on the part of the host.



(2) *Inoculation Experiments with Apple trees.*

Numerous inoculations of young Apple trees have been made with different kinds of material of *Stereum purpureum*. Sporophores from silvered Plum trees, cultivated mycelium, and spores, have been placed many times during 1911 and 1912 in branches of the following varieties of Apple trees: "Lane's Prince Albert," "Lord Suffield," and "Stirling Castle," but until the present only one such inoculation has resulted in Silver-leaf. In this particular case a branch of a bush "Lord Suffield" was inoculated in May 1912 with a piece of sporophore obtained from a silvered Victoria Plum tree and silvering of four leaves at the top of this branch, about two feet above the place of inoculation, became apparent in August. Three other branches of the same tree and four branches of another tree of the same variety were similarly inoculated at the same time but the foliage remained normal throughout the summer. It is evident that in these experiments Apple trees of the varieties named have been much less susceptible to Silver-leaf than are Plum trees. These results may be contrasted with those of Gussow<sup>(6)</sup> who finds that Apple trees in Canada are readily susceptible to Silver-leaf when inoculated with *Stereum purpureum*. Gussow does not mention the names of the varieties of Apple trees used for the purpose of inoculation. They may have been different from those used by me and this may account for the variation in results.

Upon examining inoculated branches the foliage of which has remained unaffected it is seen that the fungus has made some progress in the tissues. Thus a branch of a "Lane's Prince Albert" inoculated in February 1911 shewed in November 1911 a small zone of discoloured wood that extended two inches below the place of inoculation and an inch above it, the amount of affected wood being relatively much less than is usually the case in similar inoculations of Plum trees.

(3) *Inoculation Experiments with other kinds of Fruit Trees.*

A considerable number of young Cherry trees, chiefly of the "Florence" variety, were inoculated with *Stereum purpureum* in various forms during 1910—11 but none of these trees have become affected with Silver-leaf. Profuse gumming invariably followed the inoculations when pieces of natural sporophores from a silvered Plum tree or cultivated mycelium were used. At a later date a large number of the inoculated branches died rapidly but neighbouring branches remained

unaffected. Investigation shewed that fungus mycelium was abundant in the branches killed in this manner. This mycelium probably belonged to *Stereum purpureum* but although it frequently killed the inoculated branches with rapidity it did not pass thence into other branches.

A Gooseberry bush of the variety known as "Whinham's Industry," inoculated during 1911 with pieces of natural sporophore of *Stereum purpureum*, became silvered during 1912.

#### OBSERVATIONS ON THE MANNER OF INFECTION AND TREATMENT OF THE DISEASE.

The evidence brought forward in the present paper supports the view that in causing Silver-leaf disease in fruit plantations, *Stereum purpureum* behaves as a wound parasite, the disease being spread from one tree to another chiefly by means of spores. There is usually no lack of unprotected wounds on fruit trees and if *Stereum purpureum* is allowed to fructify in a plantation of Plum trees it is likely that Silver-leaf disease will become increasingly prevalent there. Even in fruit gardens that are well managed in other respects, fructifications of *Stereum purpureum* are sometimes allowed to develop with impunity. It cannot be too strongly urged that all tissues on which the sporophores of this fungus appear in fruit plantations should be destroyed. Experiments indicate that *Stereum purpureum* taken from material such as a dead Birch stump is equally as effective in causing Silver-leaf as *Stereum purpureum* taken from a silvered Plum tree, hence no quarter should be extended to the fungus in fruit plantations on whatever substratum it may be found.

Where Silver-leaf has appeared in a Plum plantation experience has shewn that benefit is derived by cutting out affected branches, but in order that this operation may be successful, care must be taken to cut back below the region of discoloured wood. Cases of recovery of trees which are slightly silvered are not infrequent but I see no reason to alter the suggestion previously made that Plum trees which are badly silvered and are beginning to die back, should be destroyed. Reference was made in my earlier paper to the danger of making wood piles in fruit plantations. The prevention of such accumulations is one of the first principles of plant sanitation.

The large amount of pruning and thinning out to which fruit trees are necessarily subjected may be indirectly responsible to some extent

for the malady. Even with rapidly growing Plum trees it is exceptional for a callus to grow sufficiently to protect fully the severed end of a branch from micro-organisms possessing dangerous tendencies.

Grease-banding when not properly carried out is, I think, also an influence which favours the development of Silver-leaf disease. Where the grease has been placed directly on the tree or has soaked through the band, one often sees that the bark becomes torn and dead in places. Such broken tissues offer facility for the development of *Stereum purpureum* and I have seen silvered trees on the torn bark of which *Stereum purpureum* was growing in profusion. Both for this reason and for others which are well known to fruit-growers, the grease should not be placed directly on the tree, the bands should be such that the grease cannot penetrate to the bark, and their position should be altered somewhat each autumn so that the same area of bark is not covered in successive years.

No outbreaks of Silver-leaf have been seen in which it was clear that infection had first occurred in the roots. If a tree that is badly silvered is dug up, a white mycelium is usually found attached to the roots but I have not yet seen any evidence of a tendency for it to spread outwards in the soil. This mycelium is readily traced into the discoloured parts of the wood. Of course if the trees were planted so closely that their root systems interlaced there would be abundant opportunity for infection to occur in a subterranean manner.

It has been suggested by fruit-growers that application of Ferrous Sulphate to the roots of silvered Plum trees is a means of cure, but Spencer Pickering<sup>(10)</sup> tried this method of treatment on a considerable scale at the Woburn Experimental Fruit Farm a few years ago without success.

A writer in the *Gardeners' Chronicle*<sup>(11)</sup> has recently given details of a similar method of treating silvered trees which he has found successful. The roots of silvered Plum trees were treated during 1910 and 1911 with heavy dressings of Ferrous Sulphate together with either farm-yard or complete artificial manure. In 1912 the trees thus treated shewed great improvement and one was quite cured. Only a small number of trees were treated in this way so that until the method has been tried on a larger scale it is difficult to pronounce an opinion upon it. The writer of the note was certainly hopeful of this method of treatment.

During the last two years I have had the opportunity of observing some experiments on a method of treating silvered Plum trees with

Ferrous Sulphate in a different manner. These experiments have been carried out near Wisbech by Mr E. Neaverson, who has kindly placed the following data at my disposal. Forty-nine silvered Victoria Plum trees about 20 years of age were treated in the following manner during August 1910: a hole was drilled in the trunk of each tree about three feet from the ground, the hole extending in most cases only to the outer part of the wood; about an ounce of Ferrous Sulphate was inserted in each hole which was afterwards closed with a cork bung. Of the forty-nine trees thus plugged, thirty-seven were slightly affected with Silver-leaf and the remainder were badly diseased at the time of treatment. An examination of the trees in August 1912, *i.e.* two years after treatment, shewed that twelve of the thirty-seven trees slightly infected in 1910 had recovered and were free from Silver-leaf, while none of the twelve trees badly diseased in 1910 had recovered. Thus taking all the plugged trees into consideration 25 per cent. of them had recovered while 34 per cent. of those only slightly affected have been restored to health. It is known that trees lightly attacked by Silver-leaf sometimes recover without treatment, hence it is difficult to lay much stress on the above figures, and in this particular garden one of five untreated silvered trees did recover during the period. On the other hand five of the slightly affected trees which were treated became so badly diseased during the summer of 1911 that they were felled in the autumn. Thus the above method of treating silvered Plum trees, even when only slightly attacked, does not at present appear to give much promise of success. The method is an empirical one, but it was obvious that the method should be seriously considered on account of the faith retained in it by fruit-growers. It is likely that one reason for the use of Ferrous Sulphate as a cure for Silver-leaf lies in the fact that the disease is sometimes confounded with Chlorosis, some cases of which are alleviated by applications of Iron compounds. Ferrous Sulphate placed in the trunk of a tree as in the experiments described above would certainly have a poisonous effect on mycelium present in the wood immediately around the place of application, but it is doubtful whether its influence would be extensive<sup>1</sup>.

<sup>1</sup> While this paper was passing through the press a note by Miss Baker on a new treatment for Silver-leaf disease appeared in the *Annals of Botany* for January, 1913. Miss Baker applied, both internally and externally, a concentrated aqueous extract of deliquescent fruit bodies of *Coprinus* to a silvered branch of a Victoria Plum and found that two years later this branch became almost entirely free from Silver-leaf and put forth vigorous new growth. In this note reference is made to the effect of the treatment on one tree only, so the results of its application on a large scale will be awaited with interest.

As indicating the rapid way in which Silver-leaf may spread amongst Victoria Plum trees it may be said that in the garden mentioned above, 17 trees out of 75 which were healthy in 1910 have since become affected by the disease. *Stereum purpureum* was present in abundance on silvered trees in this garden.

#### GENERAL CONSIDERATIONS AND CONCLUSIONS.

It has been shewn that Silver-leaf is a pathological condition of widespread distribution, the chief cause of the malady in the fruit plantations of this country being the fungus *Stereum purpureum*. This view is held also by Percival<sup>(8)</sup>, Spencer Pickering<sup>(9, 10)</sup>, and Güssow<sup>(5)</sup>. Examples of silvered foliage have, however, come under observation which, in my opinion, cannot be attributed to the action of *Stereum purpureum*. It is unlikely that the silvering of the leaves of seedling Plums and of such a plant as the White Dead Nettle is caused by this fungus. I look upon Silver-leaf as a general pathological phenomenon which may be caused in various ways, although at present only one of these agents, the fungus *Stereum purpureum*, is known with certainty. It appears likely that Silver-leaf may be caused also by physiological disturbances which are not connected with the action of any parasitic organism as has been suggested by Massee<sup>(10)</sup>. It will be remembered that *Stereum purpureum* living in a branch of a Victoria Plum tree may produce silvering of the leaves a considerable distance beyond the region attained by the mycelium; such action at a distance may be due to a disturbance in the transpiration current induced by the presence of the fungus below, though until the present I have been unable to confirm Percival's view<sup>(8)</sup> that the disturbing agent is an oxidase which is secreted by the fungus. Similar disturbances in the transpiration current may possibly arise from causes not associated with the action of parasitic organisms and the same phenomenon of Silver-leaf may thereby be induced. It is by no means unusual in the pathology of plants and animals for the same kind of morbid manifestation to appear as the result of different pathogenic agents, and in considering Silver-leaf a general pathological phenomenon, I look upon it somewhat in the same way as upon such a condition as, for example, hypertrophy which may be induced both by the action of various parasites and by disturbances in metabolism unconnected with the action of micro-organisms.

The views here expressed on Silver-leaf may possibly harmonise the opinions of those who have considered it to be due to *Stereum*

*purpureum* with the views of those who have placed it in the category of diseases which are not caused by the action of parasitic organisms. Thus Sorauer<sup>(12)</sup> in writing of the phenomenon known as "Milchglanz" in Germany, which is doubtless identical with Silver-leaf, describes it under the section devoted to non-parasitic diseases in his *Handbuch der Pflanzen-Krankheiten*. Delacroix<sup>(4)</sup>, who speaks of it as "le plomb" in France, also treats of the disease in the same category.

The manifestation of Silver-leaf depends, I think, partly upon leaf-structure. It is well known that certain varieties of fruit-trees exhibit this phenomenon much more than others and cases have been already mentioned in which Silver-leaf has not resulted although *Stereum purpureum* has made considerable progress in the tissues. I have seen Apple and Beech trees which have been killed by *Stereum purpureum* in all probability, but with which the phenomenon of Silver-leaf has not been associated. Thus just as the phenomenon of Silver-leaf cannot always be attributed to *Stereum purpureum*, so the destructive influence of *Stereum purpureum* is not invariably accompanied by this peculiar affection of the leaves.

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## EXPLANATION OF PLATES XI AND XII.

Fig. 1. Upper part of a silvered Victoria Plum tree, one branch of which is dead and bears fructifications of *Stereum purpureum*.

Fig. 2. Fructifications of *Stereum purpureum* on a dead branch of a Victoria Plum tree.

Fig. 3. Lower part of the trunk of a silvered Victoria Plum tree shewing fructifications of *Stereum purpureum* which developed shortly after the upper part of the tree was cut off.

Fig. 4. Cross section of a branch of a silvered Transparent Gage tree shewing diseased and healthy wood. Twice natural size.

Fig. 5. A Blenheim Orange Apple tree that has died after being regrafted with scions of "Grenadier" or "Jubilee." The scions also have died and the stock bears fructifications of *Stereum purpureum*.

Fig. 6. A Victoria Plum tree as seen one year after inoculation with *Stereum purpureum* taken from a Birch stump. The tree is dying rapidly. Fructifications of *Stereum purpureum* have appeared on the main stem of this tree since the photograph was taken.

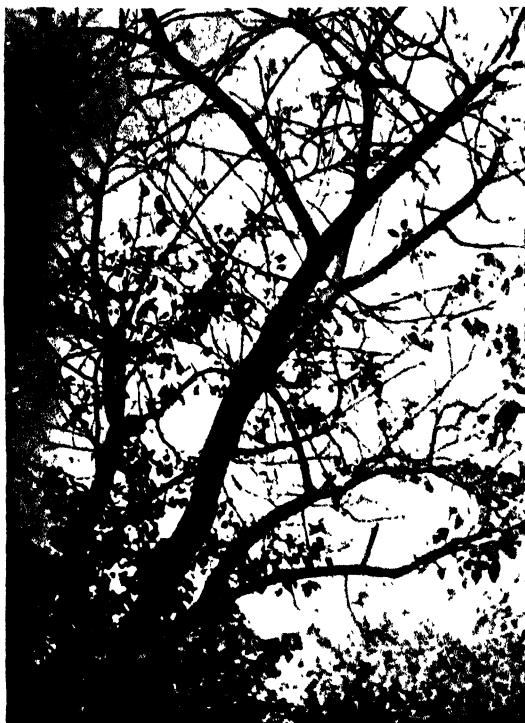


Fig. 1.



Fig. 2.







Fig. 3.



Fig. 4.



Fig. 5.

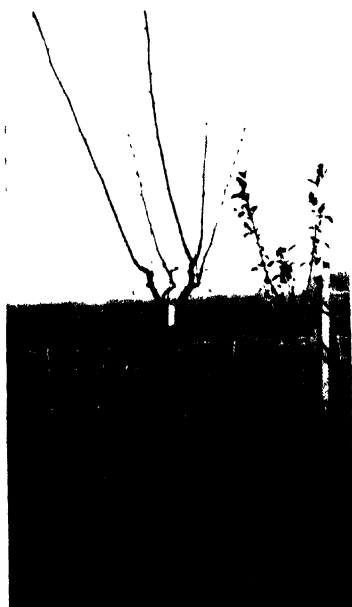


Fig. 6.



# STUDIES IN MILK RECORDS: THE INFLUENCE OF FOETAL GROWTH ON YIELD.

BY WILLIAM GAVIN, B.A.

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## *Introductory.*

IN a previous paper on the "Interpretation of Milk Records"<sup>1</sup> the following points were dealt with:

I. Selection of a figure definitive of a cow's milking capability.

II. The influences affecting such a figure.

The conclusions there reached may be summarised as follows:

(a) The usual methods of describing a cow by her total yield per calendar year, per lactation, or per average week are inconvenient both for practical breeding on a large scale and for definite enquiry on the inheritance of milk yield or its possible correlation with other characters. The figures are inconvenient because a variety of circumstances which affect them must be stated for every individual case before such figures can be reliable. Chief of these circumstances are age of cow, length of lactation, number of weeks dry before calving, interval between calving and subsequent service, and time of year of calving.

(b) It is therefore advisable to adopt some additional descriptive figure affected by the minimum number of these influences, and to estimate as accurately as is possible the effect of those influences under which it falls.

(c) The maximum day-yield three times reached or exceeded<sup>2</sup> is chosen as being the most satisfactory figure, and termed the "Revised Maximum," or R.M.

<sup>1</sup> *Journal Royal Agricultural Society*, 1912, p. 153.

<sup>2</sup> *I.e.* the highest figure common to three entries in record book, whether yields are recorded daily or weekly. What is aimed at is really the correct maximum day-yield: the stipulation that it should have appeared three times is merely to avoid the errors associated with a single sampling.

(d) This Revised Maximum bears a close relationship to the total yield of a normal lactation, and shews rather less variation than that total. The following constants were obtained:

Correlation coefficient between revised maxima and normal lactation totals  $+0.844 \pm 0.005$

Coefficient of variation.	Totals for normal lactations		...	...	...	25.72 ± 0.37
"	"	" all	"	...	...	31.69 ± 0.32
"	"	R. M. for normal	"	...	...	24.77 ± 0.36
"	"	" all	"	...	...	26.44 ± 0.25

(e) The Revised Maximum is outside two of the most active external influences, namely length of lactation, and time of service, and it is suggested that general environment has a minimum effect on it.

(f) A cow can usually be judged within a few weeks of calving, since normal lactation totals can be estimated from the Revised Maximum with considerable accuracy.

(g) The influence of length of rest before calving is negligible as regards the Revised Maximum. The latter has to be corrected however according to the age of cow, and season of year of calving.

(h) By a simple but provisional scheme of correction the mode of the extreme differences found from year to year in the Revised Maxima of individual cows can be reduced from 7 quarts to  $3\frac{1}{2}$  quarts.

It will be noticed that the Revised Maximum is referred to above as being outside the influence of:

- (1) Length of Lactation, and
- (2) Time of Service of the cow.

This contention is certainly approximately true, though in some cases the mean Revised Maximum was found to increase very slightly with length of lactation. A possible explanation of this was discussed in the paper referred to. It is thought however that the statement regarding time of service requires fuller proof than has yet been obtained, and the present paper gives the result of further work on the point.

*Time after calving at which maximum yield first occurs.*

The question as to whether the maximum day-yield can be influenced by the date of service must be dependent on whether that maximum is reached by a cow before or after the growth of the young calf within her has caused any alteration in her output of milk.

It is therefore necessary to determine firstly the time after calving at which the maximum occurs, and secondly the time after service at which foetal growth begins to reduce the milk yield,

In a preliminary examination it was found that the large majority of cows reached their maximum day-yield within a few weeks of calving, but that some of those calving about the beginning of the year did not do so until turned out to grass in the spring.

The 1421 records that were examined for this point were consequently divided into five groups according to whether the cows calved during (1) April to November inclusive, (2) December, (3) January, (4) February, or (5) March. Table I gives results obtained in actual number of cows: Table II in percentages.

TABLE I.

Date of calving	Number of cows reaching maximum day-yield during :—								Total number of cows
	1st—4th week after calving	5th—8th week after calving	9th—12th week after calving	13th—16th week after calving	17th—20th week after calving	21st—24th week after calving	25th—28th week after calving	29th—32nd week after calving	
April to November	580	249	21	3	4	1	3	1	862
December	77	38	14	1	5	6	0	0	141
January	55	38	5	17	17	4	0	0	136
February	73	26	31	43	3	0	0	0	176
March	24	33	40	7	0	0	0	0	104
Totals	809	384	111	71	29	11	3	1	1419
2 cows after 32nd week									2
									1421

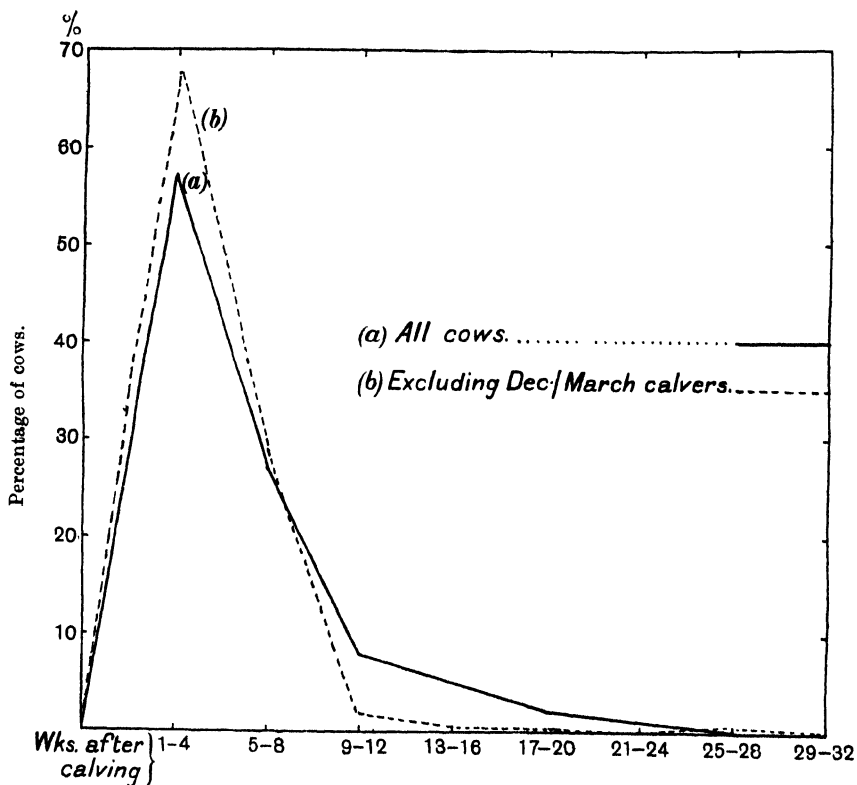
TABLE II.

Date of calving	Percentage of cows reaching maximum day-yield during :—								
	1st—4th week after calving	5th—8th week after calving	9th—12th week after calving	13th—16th week after calving	17th—20th week after calving	21st—24th week after calving	25th—28th week after calving	29th—32nd week after calving	
April to November	67·5	29	2	·5	·5	—	·5	—	100
December	55	27	10	·5	3·5	4	—	—	100
January	40·5	28	3·5	12·5	12·5	3	—	—	100
February	41·5	15	17·5	24·5	1·5	—	—	—	100
March	23	31·5	38·5	7	—	—	—	—	100
All months (Totals)	57	27	8	5	2	1	—	—	100

It will be seen that 84% of the total number of cows reached their maximum day-yield by the 8th week after calving, 92% by the 12th week, and 97% by the 16th week. Three-quarters of the 8% that had not reached their maximum by the 12th week were January and February calves, leaving only 2% for cows calving during the remainder of the year.

Taking the January and February calvers by themselves, only 72% of the former gave a maximum before the 12th week, but 97% had given it by the 20th week. February calving cows alone shew corresponding figures of 74% for the 12th week, and 100% for the 20th.

The influence of these "new-year" calvers is shewn in the following diagram, Fig. 1, which gives the curve of *all* cows compared with that obtained when December/March calving cows are excluded.



Time at which first maximum is reached.

Fig. 1.

In Fig. 2 the curves for each group in the previous table have been superimposed upon one another according to their calendar dates, and this arrangement brings out the fact that the delayed maxima are in every case ultimately reached about the same season of the year, namely April/May, when the cows respond to the extra stimulus of abundant and succulent green food.

Two cows out of the 1421, that gave their maxima after the 32nd week, have not been included in the table. One of these calved in July, and one in August, and both gave their maxima the following April when turned out to grass, in the 39th and 42nd week after calving respectively.

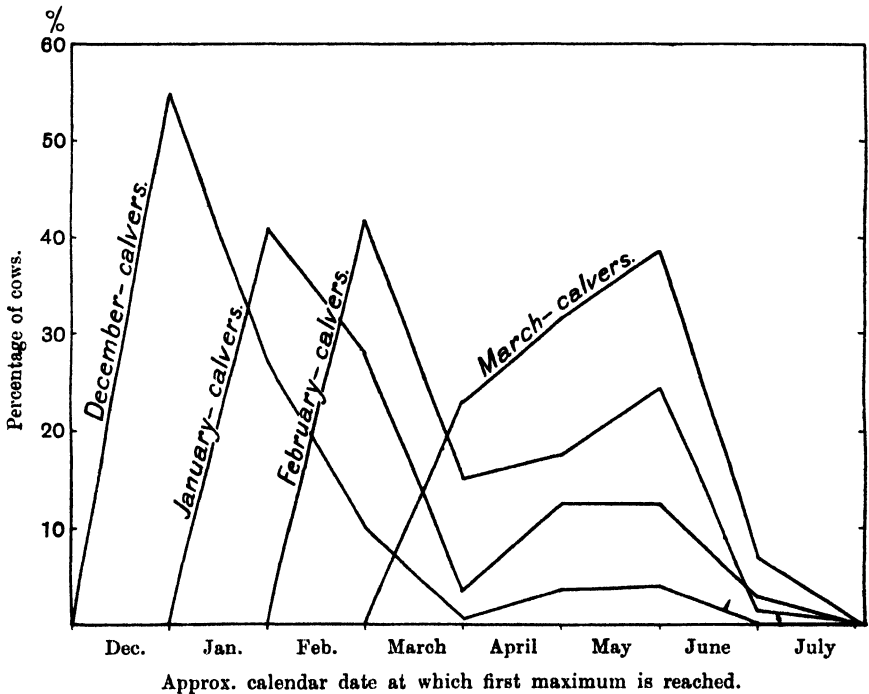


Fig. 2.

### *Influence of time of service.*

247 records of cows calving in May and June were examined. They fell into the following groups:



*Studies in Milk Records*

		<i>Time of service</i>	<i>No. of records</i>
Group I.		5th—8th week after calving	51
„ II.		9th—12th „ „	55
„ III.		13th—16th „ „	49
„ IV.		17th—20th „ „	40
„ V.		21st—24th „ „	34
„ VI.		after 36th „ „	18

[All calved between May 1st—June 30th] 247

The average daily yields in each group for the first 36 weeks after calving are given in Table III. The figures representing first and second week's yield are not reliable, as some cows were suckling calves for all or part of that time.

TABLE III.

Week after calving	I Served 5th—8th week after calving	II Served 9th—12th week after calving	III Served 13th—16th week after calving	IV Served 17th—20th week after calving	V Served 21st—24th week after calving	VI Served after 36th week after calving
	Quarts	Quarts	Quarts	Quarts	Quarts	Quarts
1	[11·6]	[9·6]	[6·7]	[9·3]	[9·9]	[8·1]
2	[13·9]	[13·3]	[12·2]	[12·1]	[12·2]	[10·4]
3	14·9	14·1	13·3	13·9	13·2	12·9
4	14·8	14·1	14·1	14·1	13·3	13·4
5	14·8	14·2	13·8	14·0	13·2	13·3
6	11·6	11·2	13·7	13·9	13·2	13·1
7	11·1	13·9	13·4	13·6	13·1	12·6
8	13·8	13·4	13·2	13·2	12·5	12·3
9	13·5	13·1	12·4	13·0	12·4	11·9
10	13·2	12·7	11·8	12·7	12·0	11·7
11	12·8	12·4	11·5	12·4	11·6	11·4
12	12·6	12·3	11·3	12·1	11·2	11·1
13	12·4	12·1	11·1	12·0	10·7	10·9
14	12·2	11·6	10·8	11·7	10·5	10·7
15	11·9	11·3	10·3	11·3	10·0	10·3
16	11·5	11·1	10·0	11·1	9·8	9·8
17	11·1	10·7	9·8	10·8	9·6	9·7
18	10·9	10·3	9·4	10·5	9·4	9·2
19	10·6	10·3	9·3	10·1	9·4	9·3
20	10·3	10·1	9·0	9·8	9·4	9·4
21	9·9	9·7	8·8	9·5	9·0	9·1
22	9·5	9·4	8·6	9·4	8·9	8·5
23	9·0	9·2	8·6	9·3	8·9	8·1
24	8·7	9·1	8·4	9·1	8·7	8·2
25	8·4	8·8	8·3	9·0	8·6	8·1
26	8·0	8·5	8·1	8·6	8·4	7·8
27	7·6	8·3	7·9	8·5	8·3	7·7
28	7·1	7·9	7·6	8·3	8·0	7·6
29	6·6	7·6	7·4	7·9	7·9	7·5
30	6·1	7·3	7·1	7·8	7·8	7·7
31	5·6	6·9	6·9	7·4	7·8	7·2
32	5·2	6·4	6·6	7·4	7·7	7·1
33	4·7	5·8	6·2	7·2	7·6	7·0
34	4·2	5·1	5·8	6·9	7·4	6·9
35	3·0	4·4	5·3	6·4	7·3	6·7
36	2·3	3·8	4·8	6·1	7·2	6·3

This somewhat unwieldy mass of figures was concentrated by dividing the 36 weeks into nine periods of four weeks each, and obtaining the average for each period. The figures are given in Table IV.

TABLE IV.

Week after calving	I	II	III	IV	V	VI
Periods	Served 5th—8th week after calving	Served 9th—12th week after calving	Served 13th—16th week after calving	Served 17th—20th week after calving	Served 21st—24th week after calving	Served after 26th week after calving
3rd and 4th	14.8	14.1	13.7	14.0	13.3	13.2
5th—8th	14.3	13.9	13.5	13.7	13.0	12.8
9th—12th	13.0	12.6	11.8	12.6	11.3	11.5
13th—16th	12.0	11.5	10.6	11.5	10.3	10.4
17th—20th	10.7	10.4	9.4	10.3	9.5	9.4
21st—24th	9.3	9.3	8.6	9.3	8.9	8.5
25th—28th	7.8	8.4	8.0	8.6	8.3	7.8
29th—32nd	5.9	7.1	7.0	7.6	7.8	7.4
33rd—36th	3.6	4.8	5.5	6.7	7.4	6.7

By calling the maximum 100 for every group, we get a more comparable series of figures shewing average yield as percentage of group maximum.

TABLE V.

Week after calving	Time of service					
	I	II	III	IV	V	VI
3rd and 4th	100	100	100	100	100	100
5th—8th	97*	99	99	98	98	97
9th—12th	88	90*	86	90	89	88
13th—16th	81	82	77*	82	77	79
17th—20th	73	73	69	73*	71	71
21st—24th	63	66	63	66	67*	64
25th—28th	52	59	58	61	63	60
29th—32nd	40	50	51	55	59	56
33rd—36th	23	34	40	47	56	51
37th—40th	—	—	—	—	48	47
41st—44th	—	—	—	—	35	45

\* Period of service.

Time after which foetal growth appears to influence yield entered in dark figures.

It would seem that in no case has foetal growth reduced yield (as compared with that shewn for the same period by groups where service has not occurred) sooner than 12/16 weeks after service.

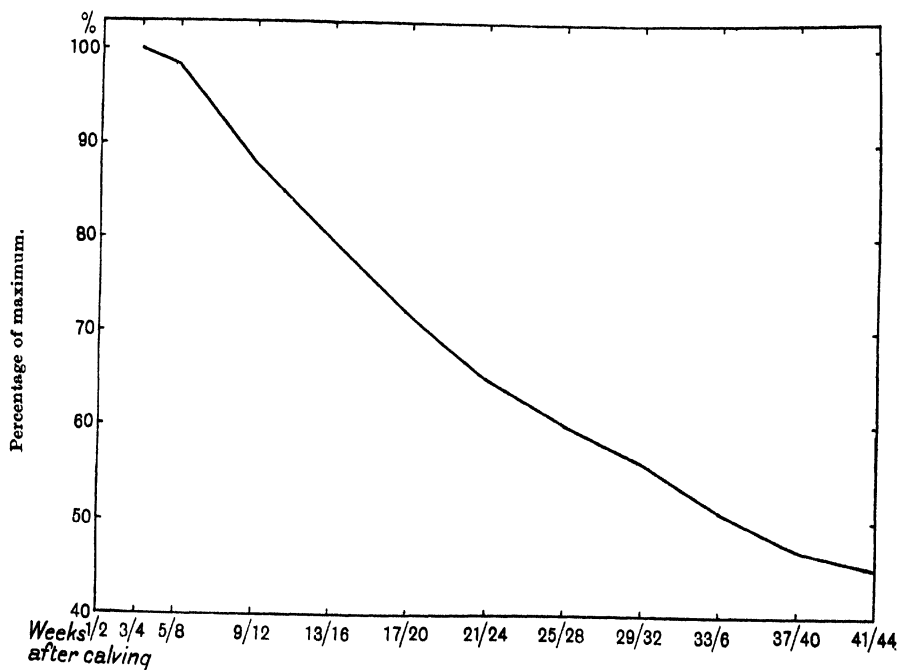
In the case of groups I, II, and V it appears to have had no influence for 16/20 weeks.

Since then 12 weeks at the very least (and probably 16/20 weeks) must elapse between *service* and any fall in yield due to foetal development, and since 97% of cows were found to have reached their maximum day-yield within 16 weeks of *calving*, and 99% within 20 weeks, the chances of the "Revised Maximum" being affected by time of service are seen to be very slight.

It is evident that the fall in yield due to foetal growth must be clearly distinguished from the fall that normally occurs in the absence of gestation.

*Fall of milk-yield where pregnancy does not occur.*

Fig. 3 gives a curve illustrating fall of milk-yield in the absence of gestation. Up to 24th week after calving it is compiled from the average of all groups, after which it follows Group V.



Fall in milk-yield of non-pregnant cows.

Fig. 3.

The cause of this gradual failing of the milk-supply does not at present seem very clear.

It is now fairly well established that the growth of the mammary gland before parturition is brought about by an internal secretion elaborated by the foetus or placenta, and that the cessation of that secretion that necessarily coincides with the expulsion of the foetus causes the breaking down of the tissue formed and the consequent production of milk<sup>1</sup>.

The institution of the milk-flow seems therefore dependent on a single and non-recurring stimulus. But having thus been once suddenly set up, it neither falls with equal rapidity, nor does it remain in constant activity, but gradually diminishes through a period of at least 6/9 months. Indeed if gestation does not intervene the lactation may be very greatly prolonged, though for commercial reasons this does not of course often occur with cows. On examining records for the present paper one cow was noticed which was sold in milk 163 weeks after calving. During this period she gave 2433 gallons, and was yielding seven quarts a day at the time of sale.

It is said that removal of the ovaries will cause lactation to continue for several years, but definite proof seems wanting that the same prolongation could not have been obtained on the same cows by constant and careful milking combined with high feeding. May not the theory have arisen through the operation removing not only the ovaries but also all possibility of gestation<sup>2</sup>?

Such cases seem to involve katabolic processes out of all proportion to the anabolism brought about by the foetal hormone before parturition, and at present we know little of any additional mechanism for maintaining the milk flow. Two points in this connection however must be mentioned.

Firstly, the removal of the products of the gland is of primary importance in prolonging its activity. It is a well-known fact that failure to empty the udder will very soon cause a cow to "dry-off." It is also supposed that the sucking of the teat<sup>3</sup> or other mechanical means

<sup>1</sup> v. Marshall, *Physiology of Reproduction*, 1910, p. 583.

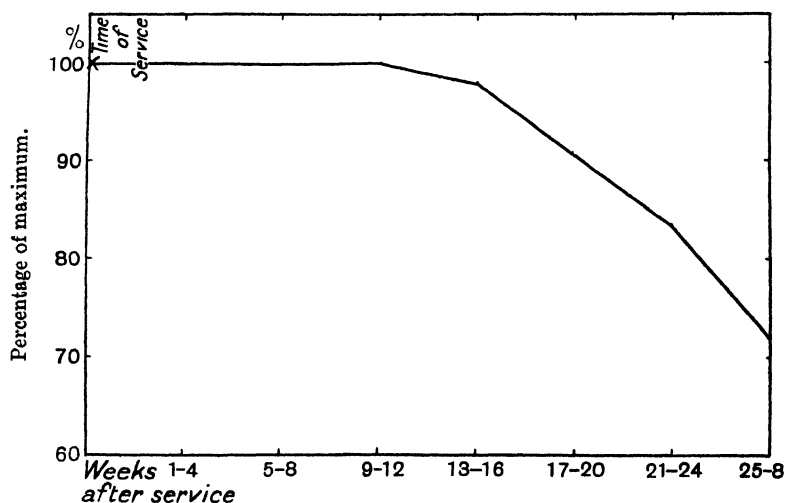
<sup>2</sup> In other cases the ovarian influence is established, as in the growth of the mammary glands at puberty in woman, which does not take place if the ovaries have been previously removed. It must be admitted that from this and other observations their influence certainly appears to be anabolic, and so in a contrary direction to milk-flow.

<sup>3</sup> Knot, "Abnormal Lactation," *American Medicine*, vol. II. 1907. Cases given of virgin girls who were nurses secreting copious supplies of milk as a consequence of allowing infants to apply this excitation. Also cases in which suckling occurred in a bull, a male goat, a wether, and in men. Quoted from Marshall (*loc. cit.*).

of withdrawing the milk<sup>1</sup> has a stimulating effect on secretion. The cessation of this excitation may also aid in the drying-off of a cow when milking is discontinued.

Secondly, it has recently been shewn<sup>2</sup> that certain organs of the body produce hormones possessing galactagogue action. The most marked results are obtained from the pituitary body, but the pineal body, corpus luteum, involuting uterus, and lactating mammary gland itself all yield extracts which, when injected into the blood-stream, cause an increased secretion of milk.

This increase, in cows<sup>3</sup> and goats<sup>4</sup> at any rate, seems to be followed by a corresponding decrease, so that the total activity of the gland is not changed. It seems probable therefore that these hormones are more directly concerned with the regulation than with the maintenance of secretion.



Fall in milk-yield due to foetal growth.  
For 28 weeks after service.

Fig. 4.

<sup>1</sup> Marshall (*loc. cit.*) gives a case where secretion was induced by repeated attempts at milking. A mare which had never had a foal could be made to yield milk by this means at any time for years.

<sup>2</sup> Mackenzie, *Quart. Journ. Exper. Physiology*, iv. 4, 1911. Schäfer, *Proc. Royal Society*, B. LXXXIV. 1911.

<sup>3</sup> Gavin, *Quart. Journ. Exper. Physiology*, vi. 1, 1913.

<sup>4</sup> Hammond, *Quart. Journ. Exper. Physiology*. In course of publication.

*Fall of milk-yield due to foetal development.*

By taking the excess of reduction shewn by groups where service occurred over that shewn by Group V, we get the following curve of diminution due to foetal growth *alone*. The data are not extensive and only the first 28 weeks after service are taken, but it is curious to note that once the reduction begins, the rate of fall due to this cause is very similar to that found where there is no gestation.

Where gestation occurs, the reduction of a cow's milk-yield is of course represented by the sum of the curves in Figs. 3 and 4, the latter coming into operation according to the time after calving at which service takes place.

These investigations are being undertaken on behalf of Lord Rayleigh and the Hon. E. G. Strutt with data accumulated by them during the last 20 years. For any deficiencies in method or treatment of the material, however, the author is alone responsible.

# THE PARTIAL STERILISATION OF THE SOIL BY MEANS OF CAUSTIC LIME.

By H. B. HUTCHINSON.

*Lawes Agricultural Trust, Rothamsted Experimental Station.*

THE practical advantages accruing from the use of lime are now generally recognised, but on account of the complexity of the changes induced it is by no means easy to determine, in the case of a field soil, the exact extent to which one effect or set of effects has contributed to the general improvement of the soil in relation to its crop-producing power.

The changes in the physical properties of a soil are accompanied by better drainage and increased aeration; the chemical action of lime leads to a liberation of plant food and the neutralisation of any acids present, and thus produces conditions more favourable to plant and bacterial growth.

Little distinction has hitherto been drawn between the effects of different calcium compounds on the soil, although such differences are plainly evident in agricultural practice. Indeed, very often the sole explanation met with is the one advanced by Boussingault, that the superiority of caustic lime over chalk lies in the extremely fine state of division in which the former is deposited as carbonate in the soil. It is the purpose of this preliminary account to indicate how essentially different this action may be and to attempt to account for such results as are frequently obtained in practice.

It has been frequently shown that when soil is treated with mild antiseptics or subjected to high temperatures, an increase of fertility ensues, and this has been attributed by Russell and the author<sup>1</sup> to a depression or destruction of soil protozoa, with a subsequent increase in bacterial growth, and production of available plant food. This effect is

<sup>1</sup> This *Journal*, vol. III, Part 2, 1909.

induced by a number of chemical compounds varying largely in composition and constitution, but fulfilling the essential requirement that the substance used should be capable of destroying protozoa, of volatilising from the soil or of being rendered innocuous to bacterial and plant growth at some subsequent period. Viewed in this light a similar effect ought to be obtained by applications of quick lime in quantities sufficient to destroy protozoa, while any excess would be converted sooner or later into carbonate, and normal growth be made possible.

Chemical and bacteriological analyses of two different soils to which various additions of calcium oxide had been made showed the correctness of this assumption.

Both these soils contained sufficient carbonate (2—3 per cent.) for all ordinary requirements, and indeed, the addition of further doses of calcium carbonate or even small quantities of oxide was without effect: when, however, the amount of oxide used was large enough to kill the larger forms of soil protozoa the general behaviour of the samples agreed closely with that of soils subjected to high temperatures or treated with different antiseptics.

When lime was applied in the proportion of 0·25—1·0 per cent. of the weight of soil, it was possible to detect an initial depression in the numbers of bacteria, and this effect persisted for a period the length of which appeared to be determined partly by the quantity of lime applied and partly by the character of the soil. In the case of a poor soil and an addition of 1·0 per cent. of calcium oxide, this depression was still evident after 3—4 months, whilst with a rich garden soil there was no trace of inhibitive action at the end of 3 weeks. Conversion of the oxide into the carbonate was followed by a period of active bacterial growth with increased production of plant food.

Pot experiments were made with these two soils and the results agree, in the main, with those obtained by bacteriological and chemical analyses of the soils.

### *Experimental.*

In March, 1911, a poor unmanured soil from Hoos Field at Rothamsted, containing 3 per cent. calcium carbonate, was passed through a 3 mm. sieve and filled into bottles in lots of 800 grams. One lot served as control and the others received 0·1, 0·5 and 1·0 per cent. calcium oxide respectively. The soils were moistened



so that the water content was about 18 per cent, and the bottles were then set aside at room temperature. Quantitative analyses for bacteria were made on nutrient gelatine, and small portions of soil were carried over to hay infusion to test for the presence of protozoa.

The results of these counts are set out in Table I together with the occurrence of protozoa. Estimations of nitrogen as ammonia and nitrates were made at the close of the experiment and showed that in all cases except that in which the soil received 1.0 per cent. calcium oxide, there had been complete conversion of ammonia to nitrates. In this one case ammonia had accumulated to the extent of 22 parts per million of the dry soil but it is not apparent whether this was formed by direct chemical decomposition of nitrogen compounds or to the action of bacteria.

TABLE I. *Showing the effect of additions of Calcium Oxide on numbers of Bacteria and Ammonia and Nitrate production.*

Soil and Treatment	Protozoa in soil after treatment	Bacteria (in millions) per gram of dry soil after					Ammonia and Nitrates, parts per million of dry soil after 250 days		
		7 days	18 days	140 days	200 days	250 days	Ammonia	Nitrate	Total
Untreated soil	Vorticella, Colpoda, Amoebae and Monads	12.1	11.55	—	—	12.9	nil	17.4	17.4
Soil + 0.1 % CaO	Vorticella, Colpoda, Amoebae and Monads	8.4	15.3	16.5	15.3	15.2	nil	19.6	19.6
Soil + 0.5 „	Amoebae and Monads	2.4	18.3	125.3	157.5	70.8	1	52.1	53.1
Soil + 1.0 „	„ „	1.1	3.4	61.3	444.0	300.4	21.5	7.8	29.3

Just as in the case of soils treated with toluene, chloroform, and similar antiseptics, so in this case the death of the larger protozoa is accompanied by increased bacterial growth<sup>1</sup> and plant food production. The bacterial content of the heavily limed soils is considerably higher than is usually obtained by the use of mild antiseptics and might be attributed to a combined partial sterilisation effect and a more or less far-reaching chemical decomposition of organic nitrogen compounds in the soil. Further evidence of this action will be adduced later on.

<sup>1</sup> Since going to press, the author has found similar increases in bacterial numbers recorded by Fischer (*Landw. Versuchs-Stat.*, 1909, **70**, p. 335), who attributes them to stimulation effects.

It would appear from these data that caustic lime exercises initially a distinct depressive action on the growth of bacteria when applied to the soil in quantities greater than 0.1 per cent., but the intervals between the times of analyses in the early stages are too long to allow of any deduction as to the rapidity of the removal of caustic lime in this case.

In order to ascertain the rapidity of this change to carbonate, a second series of experiments was started in November 1911, with a similar arable soil and also a rich garden soil containing 2 per cent. calcium carbonate. The treatment was similar to that of the previous series, but estimations of bacteria were made during the period in which initial depression and subsequent recovery of bacterial growth were thought to take place.

The results contained in the following Table show how very different these soils are in their behaviour to treatment.

TABLE II. *Showing the effect of additions of Calcium Oxide to Arable and Garden Soils.*

Soil and Treatment	Protozoa in Soil after treatment	Bacteria (in millions) per gram of dry soil after			Ammonia and Nitrates, parts per million of dry soil after 83 days		
		19 days	55 days	83 days	Ammonia	Nitrate	Total
<i>Arable Soil</i>							
Untreated Soil	Colpoda, Amoebae and Monads	5.8	10.8	10.9	1.4	17.4	18.8
Soil + 0.1% CaO	“ “ “	8.8	11.1	10.9	1.4	19.3	20.7
Soil + 0.5 “	“ “ “	10.9	40.9	64.5	15.0	18.0	33.0
Soil + 1.0 “	Amoebae and Monads	1.5	1.7	1.5	23.2	11.7	34.9
<i>Garden Soil</i>							
Untreated Soil	Colpoda, Amoebae and Monads	12.8	15.6	8.6	1.8	17.4	19.2
Soil + 0.1% CaO	Hypotricta, Colpoda, Amoebae and Monads	14.3	31.3	11.0	2.0	28.8	30.8
Soil + 0.5 “	Amoebae and Monads	202.0	122.0	103.0	1.9	66.0	67.9
Soil + 1.0 “	“ “	99.2	454.6	498.6	40.6	21.3	61.9

The addition of 0.1 per cent. calcium oxide to the arable soil was without effect on the numbers of bacteria throughout the course of the experiment, and failed to induce any increase in the ammonia and nitrate nitrogen. Heavier applications of 0.5 per cent. oxide gave rise to increased bacterial growth within the first 55 days and this continued for at least 83 days, whilst 1.0 per cent. exerted an inhibitive

effect for the whole period. In spite of the low bacterial content in the latter case, there had occurred an accumulation of free ammonia thus indicating considerable chemical action of the lime itself.

As regards the garden soil, attention may be drawn to the transitory effect produced by small applications of lime. Here there occurred an initial rise of bacteria to upwards of 30 millions per gram and a subsequent drop to the normal content of the soil. Heavier doses would appear to be rendered innocuous to bacteria within the first 19 days and give rise to a phenomenal increase in the numbers of bacteria, which, particularly in the case of the soil with 1.0 per cent. oxide, is so much above that normally found in a soil treated with chemically inert volatile antiseptics.

These high bacterial numbers might possibly be due to direct chemical action, resulting in a liberation of simpler carbonaceous compounds, which alone would lead to increased bacterial growth. On the other hand, it might be attributed to the survival and subsequent increase of certain bacteria which are normally killed by treatment with toluene. It is often found that inoculation of a tolued soil with an extract containing the bacteria of an untreated soil, results in a much higher bacterial content, and a greater amount of chemical change, than is attained in the tolued soil alone, due no doubt to the growth of more active but less resistant species of bacteria.

Similarly, nitrifying organisms are always killed by toluene but would appear to remain alive in these limed soils in the majority of cases, or to find their way into the soil from the sides of the bottle, since it is impossible to sterilise it so efficiently by the use of lime as it is by means of heat or volatile antiseptics.

In any case, however, the death of protozoa causes a liberation of nitrogenous material not normally available and this in itself would lead to increased bacterial growth; that such a liberation does take place is shown by experiments carried out in the following manner: Portions of 500 grams of arable soil were mixed with calcium oxide in the proportion of 0.1, 0.25, 0.5 and 1.0 per cent. and thoroughly agitated with 500 c.c. of distilled water and allowed to stand at laboratory temperature for 24 hours. The soil suspension was then filtered, the filtrate being taken for titration and nitrogen estimations, whilst the residue was examined for bacteria after 6 and 22 days.

The amount of extractable nitrogen in this soil would appear to be in direct relation to the weights of lime used, in fact, the curve plotted from these data is quite linear in character. No decided change

in the reaction of the filtrate occurs except in the case of the heaviest dressing of lime, although the slight change from an acid to an alkaline reaction of the filtrate is also accompanied by higher bacterial numbers after a period of incubation.

TABLE III. *Showing the liberation of soluble Nitrogen Compounds from the Soil by Caustic Lime.*

	Percentage of Calcium Oxide added to Soil				
	Control	0.1 %	0.25 %	0.5 %	1.0 %
Soluble nitrogen in filtrate (excluding nitrates)	1.52 mgrm.	3.80 mgrm.	5.32 mgrm.	8.36 mgrm.	9.88 mgrm.
Titration of filtrate 25 c.c.	= 0.05 c.c.	= 0.05 c.c.	= 0.05 c.c.	= 0.1 c.c.	= 4.2 c.c.
	N 10 NaOH	N 10 NaOH	N 10 H <sub>2</sub> SO <sub>4</sub>	N 10 H <sub>2</sub> SO <sub>4</sub>	N 10 H <sub>2</sub> SO <sub>4</sub>
Protozoa	Colpoda, Amoebae, Monads	Colpoda, Monads	Colpoda, Monads	Monads	Monads
Bacteria (in millions) per gram of soil after					
6 days	15.8	30.5	29.7	31.7	19.6
22 days	22.3	32.1	69.8	54.2	68.2

The bacterial counts in all the treated soils show a uniform rise after six days, with the exception of the sample receiving 1.0 per cent. of lime in which recovery from an initial depression would appear to be just taking place. After 22 days the bacteria in the soil receiving 0.1 per cent. have scarcely increased, whilst those in the other three samples have multiplied considerably.

As regards the bacterial growth, therefore, more favourable conditions occur with the addition of 0.25 per cent. than with 0.1 per cent. oxide, but whether this is a permanent increase or merely a temporary rise to be followed by a fall in numbers, such as is often met with in soils incompletely freed from the larger protozoa, is a point that can only be decided by further tests.

Somewhat similar experiments have been carried out with an acid soil from the Woburn Experimental Station<sup>1</sup>, and also a rich soil (containing about 1.0 per cent. calcium carbonate) from the Chelsea Physic Gardens. In this case 100 grams of soil were mixed with various quantities of oxide and 50 c.c. of distilled water, and after

<sup>1</sup> This soil was obtained from one of the Woburn plots which have been rendered distinctly acid by continuous applications of ammonium sulphate, and was kindly placed at the disposal of the author by Dr J. A. Voelker, Director of the Station.

shaking, the samples were allowed to stand for 4 hours and then filtered. The residuc was washed with a further quantity of 50 c.c. of water, and the whole of the filtrate taken for nitrogen estimations.

TABLE IV. *Showing the action of Caustic Lime on the liberation of soluble Nitrogen Compounds and on Bacterial growth.*

	Woburn Soil		Chelsea Soil	
	Nitrogen in 100 c.c. of filtrate	Bacteria (in millions per gram of soil) after 3 days	Nitrogen in 100 c.c. of filtrate	Bacteria (in millions per gram of soil) after 3 days
Control	0.95	9.7	0.55	22.2
0.1 % CaO	0.80	16.5	—	27.0
0.25 „	1.45	71.4	0.90	23.9
0.5 „	2.50	6.2	3.45	13.0
1.0 „	2.70	2.5	2.35	3.9

The washings of the Woburn soil resemble, with respect to soluble nitrogen, those obtained from the Rothamsted arable soil.

The effect of supplying calcium oxide in doses of 0.25 and 0.5 per cent. is to increase markedly the amount of extractable nitrogen and this is not appreciably raised by using double the amount of lime. On the other hand, the greater part of the lime in the case of the Woburn soil receiving 0.25 per cent. would appear to have entered into combination or to be extracted by the wash-water employed, thus leaving the soil in a favourable condition for bacterial growth and resulting in the relatively high content of 71.4 millions per gram. Heavier applications reduce bacterial numbers greatly and would doubtless continue to exercise this effect for some considerable period in the case of such a light soil as the one under consideration.

Comparable results were obtained with the Chelsea soil. On account of the presence of a sufficiency of calcium carbonate in the soil, the addition of lime up to 0.25 per cent. was without any decided effect either on the amounts of soluble nitrogen or bacteria, but greater doses lead to increased liberation of nitrogen and decreased initial bacterial numbers.

#### *Pot Experiments.*

Since the foregoing results appeared to indicate an action of caustic lime which had not previously been recognised, it was considered

desirable to carry out similar experiments in pot culture with the same arable and garden soils as were used in earlier laboratory tests. Glazed pots containing 18 lbs. and 20 lbs. of arable and garden soils respectively were used, and lime was added in the proportion of 0·1 per cent., 0·5 per cent. and 1·0 per cent. With each of the soils a series of pots was included where an addition of calcium carbonate equivalent to 0·5 per cent. of the oxide was made, in order to show that the results obtained were not due to any increase of carbonate in the soil, but rather to a specific action of the lime in the caustic state.

The mixtures of soil and lime were allowed to stand for three weeks under cover, at the end of which time they were turned out, the reaction of the soil tested with litmus paper, and wherever distinctly alkaline, carbon dioxide was passed through. Only in one case, namely, that of the poor arable soil with 1·0 per cent. oxide, was the soil alkaline and a strong smell of ammonia was to be detected.

Ten barley seeds were sown in each pot on May 2, 1912, and germination was regular in all soils except in those which had received 1·0 per cent. oxide, where a slight but noticeable retardation was apparent; this effect persisted for some considerable time especially in the case of the arable soil.

On the whole the addition of caustic lime caused growth entirely characteristic of plants growing in partially sterilised soils: the plants were stunted and of a dark bluish green colour. The crop was cut on August 14th, and the average weights of dry produce per set of three pots are given in Table V. After the barley roots had been taken out a fresh crop of mustard was sown on August 21st, and showed greatly increased growth in those soils which had received lime in quantities sufficient to induce partial sterilisation effects in laboratory experiments. Photographs of the crops in garden soils are shown on Plate XIV.

It is difficult to account satisfactorily for the uniform depression shown by the first crop in garden soils with each addition of caustic lime. Bacteriological analyses of these soils, made before the barley was sown, showed that an appreciable increase in the numbers of bacteria had already taken place, and thus indicated that much if not all the oxide applied had been converted to the mild form. Furthermore, in the case of the arable soil there was no such depression with the two applications of 0·1 and 0·5 per cent. of oxide; decreased growth in the soil with 1·0 per cent. of oxide is not surprising when one bears

in mind the persistence of caustic lime in this soil, as shown by earlier bacteriological and chemical analyses.

It is proposed to extend these studies to various types of soils with a view to ascertaining, if possible, what applications of lime are necessary to induce partial sterilisation in each case, the rapidity of conversion of the lime from the caustic to the mild form, and the nature of the injurious action on crops where such is evident. In the consideration of these results and their relation to field conditions it should be remarked that in converting percentages of lime into tons per acre, an addition of 0.1 per cent. to a soil is almost equivalent to a dressing of one ton per acre calculated on the top nine inches of soil. Since, however, the effective layer, as far as numbers of bacteria are concerned, is constituted by the top six inches, the quantities used in these experiments would be about 0.6, 1.6, 3.3 and 6.6 tons per acre. The latter dressings would certainly appear to be in excess of those generally advised and adopted in farm practice, but this does not necessarily mean that they are too high, whilst the scarcity of data showing the effect of various quantities of caustic lime on arable land precludes any attempt at correlation.

On the other hand, the amounts applied about the middle of last century varied from 3—10 tons per acre, according to the state and character of the soil, in order to produce maximum results. Were these maximum results due, in any degree, to partial sterilisation of the soil?

TABLE V. *Showing the Results of pot experiments with Untreated and Limed Soils.*

Treatment	Arable Soil			
	Dry produce per pot (average yield of three pots)			
	1st crop, Barley		2nd crop, Mustard	
	in grams	relative weight	in grams	relative weight
Untreated Soil	9.5	100	0.55	100
Soil + 0.9 % $\text{CaCO}_3$	11.1	117	0.55	100
Soil + 0.1 % $\text{CaO}$	12.4	130	0.50	91
Soil + 0.5 "	15.4	162	1.16	211
Soil + 1.0 "	3.8	40	3.88	705

Treatment	Garden Soil			
	Dry produce per pot (average yield of three pots)			
	1st crop, Barley		2nd crop, Mustard	
	in grams	relative weight	in grams	relative weight
Untreated Soil	28.6	100	2.28	100
Soil + 0.9 % $\text{CaCO}_3$	28.4	99	1.86	81
Soil + 0.1 % $\text{CaO}$	24.7	86	1.92	84
Soil + 0.5 „	24.5	86	5.51	242
Soil + 1.0 „	24.6	86	9.50	417

### *Conclusions.*

When a soil is treated with lime, either in the caustic or mild form, an improvement of its physical condition results; the treatment gives rise to a certain amount of chemical action with a liberation of nutrient substances, and also, by neutralising any acids present, provides a more favourable environment for the various classes of organisms existing in the soil.

This in itself is not sufficient to account for many of the results that are obtained in practice. Caustic lime has a recognised value as an antiseptic and, when applied to the soil, even in the presence of large quantities of calcium carbonate, has a pronounced effect in disturbing or even destroying the state of equilibrium normally existing between the micro-flora and the micro-fauna of the soil.

The action of caustic lime has been found to be intermediate in character between that exercised by volatile antiseptics and the changes induced by high temperatures. In addition to killing many bacteria and causing the death of the larger protozoa, which would appear to exert a depressive action on the growth of bacteria, it brings about a decomposition of the organic nitrogenous constituents of the soil. It is highly probable that these decomposition products serve as nutrients for bacteria and are subsequently resolved into plant food.

The depression of bacterial activities in soils treated with caustic lime would appear to persist until all the oxide has been converted into carbonate; this is followed by a period of active bacterial growth and



increased production of plant food. The inhibitory action of caustic lime on soil bacteria varies with the soil and is possibly governed by the organic matter present.

In the main, pot experiments give results similar to those obtained in the laboratory by bacteriological and chemical analyses. A poor arable soil, containing a sufficiency of calcium carbonate, gave increased yields when treated with 0·5 per cent. of calcium oxide. A rich garden soil, on the other hand, gave decreased yields in the first crop but largely increased yields in the second crop. The conditions leading to this depression are not clear at present and confirmatory experiments are necessary before any explanation can be attempted. It is, perhaps, worthy of note that similar depression is often observed with soils that have been subjected to high temperatures.



Fig. 1.  
First Crop: Barley.



Fig. 2.  
Second Crop: Mustard.

Experiments on the action of Caustic Lime on Garden Soil. Treatment: C.R. untreated soil. Nos. 14, 17, 20, and 23 soil treated with 0.9 per cent. calcium carbonate, and 0.1, 0.5 and 1.0 per cent. calcium oxide respectively.



# OBSERVATIONS ON THE FAT GLOBULES IN MILK.

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## INTRODUCTION.

IT is a well-known fact that creams vary in the process of churning; it is known that some creams take longer than others to "come"; it is known also—and this is of far greater importance—that the butter obtained varies very greatly, both in quantity and quality. But the reasons for these variations are not known. The question arose, some time ago, whether any function of the globules of fat (size, number, etc.) influenced this variation.

This point was taken up by us, at the request of Mr Ernest Mathews, in 1909, and has formed the subject of much work in the laboratory at Watford.

That the globules do have an effect upon the churnability is shown by several writers. For instance, Sturtevant (*Ann. Report, U.S.D.A.*, 1880; Abridged Account in *Journ. Royal Agric. Soc. Eng.*, 1882, XVIII, (2), 475—495) showed that "the larger the globule, other things being equal, the quicker the churning." He states that a milk with larger globules gives better butter, and also that "churning is a physical process and acts upon the larger globules only."

It has been stated by Klusemann (*Inaugural Dissertation*, Leipzig, 1893; Abst. in *Chem-Zentralblatt*, 1894, (1), 646; *U.S.D.A. Expt. Stat. Record*, v, 1022) that butter made from milk with large globules has a deep yellow colour, a good taste, and is hard; whereas that made from a milk with small globules has a whitish colour, and is inferior in taste and consistency. He concludes also that the greater the proportion of large globules, the more completely can the cream be churned. But no information is given as to whether this observation is made upon milk

from cows of the same breed; for it is very well known that the breed has a great influence on the colour of the butter.

At the outset, the problem did not appear to be very difficult; but as the work progressed, it was obvious not only that the experimental work was by no means simple, but also that the results were very inconclusive. At first, consideration was given to the globules solely; but it was soon realised that a large number of other factors affected the problem. Moreover, it was quite impossible to give consideration to one variant only, so that it was difficult to determine to what factor any particular result might be due.

Of the variable factors which might affect "churnability<sup>1</sup>," the following are the chief:—

- (a) The physical properties of the milk.
- (b) The size, number and distribution of the globules.
- (c) The condition of the serum immediately surrounding the globules—a covering or membrane.
- (d) The effect of food, whether on the globules or on the serum.
- (e) The constitution of the serum.
- (f) The temperature of the cream.
- (g) The ripeness of the cream.
- (h) The percentage of fat in the cream.
- (k) The breed of cow.
- (l) Period of lactation.
- (m) The influence of bacteria.

Before it is possible to determine whether any variation is due to a function of the globules themselves, it is necessary to ascertain the general effect of the other factors.

The size, number and distribution of the globules have been considered in a previous paper (*Journ. Agric. Sci.*, iv, 1911, Pt. 2, 150—176); further results are given below (Tables V—XIII, Figs. 3—6), and a résumé is given of the present knowledge of the much debated problem of the membrane surrounding the globule. The effect of the feed upon the globules forms the main portion of this communication, but its effect on the serum has not been investigated. The constitution of the serum, the effect of the temperature of the cream and the percentage of fat in the cream, upon the churnability, have been considered and will be discussed later. Attention has not yet been given to other factors.

<sup>1</sup> The term "churnability" is used in a general sense to include all the variations which occur in churning.

## SUMMARY OF PREVIOUS WORK.

Results of our previous work were published in the *Journ. of Agric. Sci.*, iv, 1911. There, also, a brief summary of the chief papers published on the subject of the fat globules was given, to which it is unnecessary to refer in detail here<sup>1</sup>.

When the work was first commenced in 1909, the problem before us was the consideration of the variation in the size of the fat globules, with relation to churning, as regards the *different breeds* of cattle. The breed was considered because it was usually supposed that this was one of the chief factors which influenced churning. The most definite result of our work was that it was shown that consideration must be given to *the character of the milk, irrespective of the breed*. This conclusion is quite contrary to that of other workers, but an examination of their figures shows, undoubtedly, that this is actually the case: the results given by Woll (Digestion Expts., *Seventh Annual Report, Agric. Expt. Stat., Wisconsin*, 1890, 238; also *Agric. Sci.*, 1892, vi, 445) emphasise this point particularly. It is also shown by this year's work, as may be seen from Tables V—XI.

The comparative size of the globules has been worked out very thoroughly by Gutzeit, who measured the mean volume of the globule. Other workers give their results as "relative sizes." In our work we attempted to ascertain the distribution of the fat in the globules, and to this end the number of globules of each size was determined, and curves were drawn. The result of this, however, was negative. At that time we were considering the *breeds of the cows* from which the milks were obtained; had we considered them solely as milks of a certain mean size of globule, much more might have been achieved.

An apparatus was devised to give an absolute figure for the churnability of any cream, but, until the effect of the other factors has been determined, it is impossible to interpret the results. Some experiments to ascertain the optimum temperature were described also.

## CONSTITUTION OF THE SERUM.

In addition to the globules themselves, one must consider the causes which prevent them from coalescing. This may be due to

<sup>1</sup> In the English literature, where the subject of globules is dealt with, reference is given chiefly to D'Hont's paper ("Essai sur les dimensions du globule gras en suspension dans le lait. Influence de la race." Courtrai, 1890). This is remarkable since a work has been published by Gutzeit (*Landwirtsch. Jahrbücher*, xxiv. Berlin, 1895, 539—668) which is very exhaustive, and which is based on a very large number of actual measurements.

some quality of the serum in which they are floating. For instance, Babcock (*New York Expt. Stat., Fifth Report, 1887*) investigated the viscosity of various milks, and found that the viscosity of milk is greatest with a large mean-size globule. Of the constituents of milk serum, the albuminoids have by far the greatest influence on the viscosity. He states that "a low coefficient of viscosity for the serum is most favourable to the economical production of butter." On the other hand, coalescence may be prevented by some covering round the globules. This point is considered later.

In the work of 1910, published in the *Journ. of Agric. Sci.*, analyses were made to determine whether any correlation existed between the proteids and sugar in the serum, and the fat and the size of globule in the milk. No definite conclusion can, however, be drawn.

Hart (*Journ. Amer. Chem. Soc.*, xxx, No. 2, 1908, 281—285), who investigated this point, showed that:—

(1) The relation of casein to fat is variable.

(2) It is due, chiefly, to individuality of the cows.

Brevans (*Hyg. Viande et Lait*, III, 1909, No. 12, 593) also gave tables to show the effect of individuality.

In 1911, therefore, consideration was given to the nitrogen-containing constituents. Analyses were made upon those milks, the creams of which were used for the churnability and for optimum temperature experiments. The total nitrogen was determined by Kjeldahl's method; the total proteids by precipitation with tannic acid; the casein by precipitation with magnesium sulphate; the albumen by difference. The results are given in Table I.

TABLE I.

		Jersey		Guernsey		Kerry		Red Poll	
		Whole milk	Cream	Whole milk	Cream	Whole milk	Cream	Whole milk	Cream
Per cent. fat			58.21	4.10	57.91	3.92	57.08	3.97	57.91
" N. $\times 6.38$			1.50	3.34	1.42	3.29	1.53	3.31	1.57
" non-proteid N.			0.22	0.22	0.01	0.28	0.11	0.18	—
" total proteids			1.28	3.12	1.41	3.01	1.44	3.13	1.56
" casein			1.14	2.37	1.17	2.49	1.105	2.62	0.88
" albuminoid (?)			0.14	0.75	0.24	0.52	0.32	0.51	—
Total proteids									
Total N. $\times 6.38 \times 100$			85.3	93.4	99.4	91.6	93.7	94.4	99.6
Casein									
Total proteids $\times 100$			89.4	76.1	83.1	82.6	76.8	83.9	56.5

No correlation could be found between these figures and those obtained with the churnability apparatus and the other determinations which were made; this is probably due to the large number of factors involved.

### OPTIMUM TEMPERATURE.

It is known that better results are obtained by churning at a low temperature; but, when the literature was examined in order to see upon what experiments this knowledge was based, it was impossible to find any *definite* data.

Some excellent tests were carried out at the various shows of the Royal Agricultural Society, but no precautions were taken to keep the temperature constant. Indeed, on examining some of the results—excellent *practical* results—one finds that the temperature varied, in some cases, between 54° F. and 60° F.; that is to say, practically over the whole range of churning temperature. These were *practical* tests, and it is difficult to see how they could have been carried out differently, without entirely modifying the scale and the method.

In this connection, Robertson (*Canada Expt. Farms Report*, 1892, 71—78; Abst. in *U.S.D.A. Stat. Record*, v, 1894, 641) carried out some experiments on sweet cream at temperatures ranging from 41° F. to 58° F. at the *beginning* of churning. The temperature at the *conclusion* of churning was from 57° F. to 62° F., so that here also no precautions were taken to keep the temperature constant. His results showed that:—

(1) With an initial temperature of 50° F., or under, the quantity of the fat remaining in the butter-milk need not exceed 0.25 %.

(2) For the efficient conversion of the fat, the temperature of the cream should not be above 50° F. *at the commencement*.

(3) That the churn (if a revolving one) should not be more than one quarter full.

Robertson made experiments also on the addition of water to the cream, and showed that there was slightly less percentage-conversion where water had been added to the cream before ripening. He showed, moreover, that the period of lactation had an influence on the conversion, inasmuch as the loss of fat was greatest with the milk of cows which had been more than six-and-a-half months in milk.

In 1909 an attempt was made to determine the optimum temperature definitely, and the apparatus and method were described in detail in the *Journ. Agric. Sci.*, iv, Pt. 2, 1909, 167. It was found that



at higher temperatures the percentage of the fat taken in the cream, which was converted into butter, varied.

The work was repeated in 1911, and in this case not only the temperature but also the percentage of fat in the cream was varied. It seemed to be desirable that the percentage of fat in the cream should be as near as possible to that taken in practical work. Seven samples of cream were taken, as prepared ready for churning by seven different dairy-maids. The fat was found to vary from 36% to 45.5%, the average being 38.5%. A cream containing 38% fat would not churn in our apparatus, however; 30% was the maximum, and even in that case it was necessary to add "breaking" water. Experiments were made, therefore, using fresh cream containing 30% and 25% of fat respectively. 200 grams were taken of each cream, and these were churned at 54° F., 58° F. and 62° F. Where "breaking" water was added towards the end of the churning, the amount was noted. The butter-milk was weighed, and the percentage of fat in it was determined; from this the percentage of fat lost in the butter-milk was calculated.

The results are shown in Tables II and III, and are plotted in curves in Figures 1 and 2.

These results were obtained from one series of experiments only; for at that time the various breeds were being considered, and these could only be obtained at the Royal Show, where it was impossible to make duplicate experiments. Now that it is realised that the milks should be considered in respect to the size of their globules, rather than to the breed, it is possible to repeat the experiments in the laboratory. This it is proposed to do in the near future.

The most definite conclusion which can be drawn from these experiments is that the percentage of fat in the cream has a very marked influence upon the percentage conversion of fat into butter.

It is obvious that the temperature has a considerable influence, but to what extent, these results can only afford an approximation.

The effect of temperature is not marked in the case of Jersey, Guernsey or Kerry milks, provided that the cream is thick. In the other breeds, the effect is more pronounced; but in these cases better results might have been obtained if the cream had contained more fat. In all cases, however, the effect of temperature is very marked if the cream is too thin.

In the tables opposite, the names of the breeds are given, but it must be remembered that the results, as shown, are not necessarily any criterion as to the suitability of any particular breed for dairy work. This point is considered more fully on p. 356.

TABLE II.

Breed	Mean Dia.	Temp. Deg. F.	% Fat	Cream taken gms.	Fat taken gms.	Water added gms.	Weight of Butter obtained gms.	Weight of Butter-milk gms.	% Fat in Butter-milk	Weight of Fat in Butter-milk gms.	% Fat lost
Shorthorn		54	29	200	58	( 70	68	202	0.75	1.5	2.6
		58				( —	66.2	133.8	2.3	3.1	5.3
		62				( —	63.8	136.2	4.4	6.0	10.3
Jersey		54	30	200	60	( 130	72.5	257.5	0.1	0.25	0.4
		58				( 100	72.0	228.0	0.2	0.45	0.75
		62				( 70	78.7	191.3	0.3	0.6	1.0
Guernsey	3.09	54	30	200	60	( 70	68.8	201.2	0.2	0.4	0.7
		58				( 70	69.4	200.6	0.2	0.4	0.7
		62				( —	72.0	128.0	0.45	0.6	1.0
Red Poll	2.84	54	30.5	200	61	( —	70.0	130.0	2.25	2.9	6.0
		58				( —	69.0	131.0	3.8	4.9	8.0
		62				( 70	70.1	199.9	5.58	11.1	18.2
Kerry	2.88	54	30	200	60	( 70	73.2	196.8	0.1	0.2	0.3
		58				( 70	68.8	201.2	0.4	0.8	1.3
		62				( 70	70.2	199.8	0.5	1.0	1.6
Ayrshire		54	30.5	200	61	( 70	74.2	195.8	0.5	1.0	1.6
		58				( 70	77.2	192.8	0.75	1.4	2.3
		62				( —	75.6	124.4	2.6	3.2	5.2

TABLE III

Breed	Mean Dia.	Temp. Deg. F.	% Fat	Cream taken gms.	Fat taken gms.	Water added gms.	Weight of Butter obtained gms.	Weight of Butter-milk gms.	% Fat in Butter-milk	Weight of Fat in Butter-milk gms.	% Fat lost
Shorthorn		54	25	200	50	( —	61.5	138.5	2.2	3.0	6.0
		58				( —	41.7	158.3	10.3	16.3	32.6
		62				( —	49.5	150.5	9.9	14.9	29.8
Jersey		54	25	200	50	( 50	64.8	185.2	0.4	0.7	1.4
		58				( —	51.7	148.3	5.4	8.0	16.0
		62				( —	44.2	155.8	6.9	10.7	21.4
Guernsey		54	26.75	200	53.5	( —	66.0	134.0	1.2	1.6	3.0
		58				( —	51.7	148.3	7.7	11.4	21.3
		62				( —	43.7	156.3	8.1	12.6	23.5
Red Poll		54	26.75	200	53.5	( —	63.7	136.3	3.9	5.3	10.0
		58				( —	59.1	140.9	10.1	14.2	26.5
		62				( —	55.6	144.4	10.8	15.6	29.2
Kerry		54	25	200	50	( —	61.5	138.5	1.2	1.6	3.2
		58				( —	52.3	147.5	2.7	4.0	8.0
		62				( —	48.5	151.5	9.9	15.0	30.0

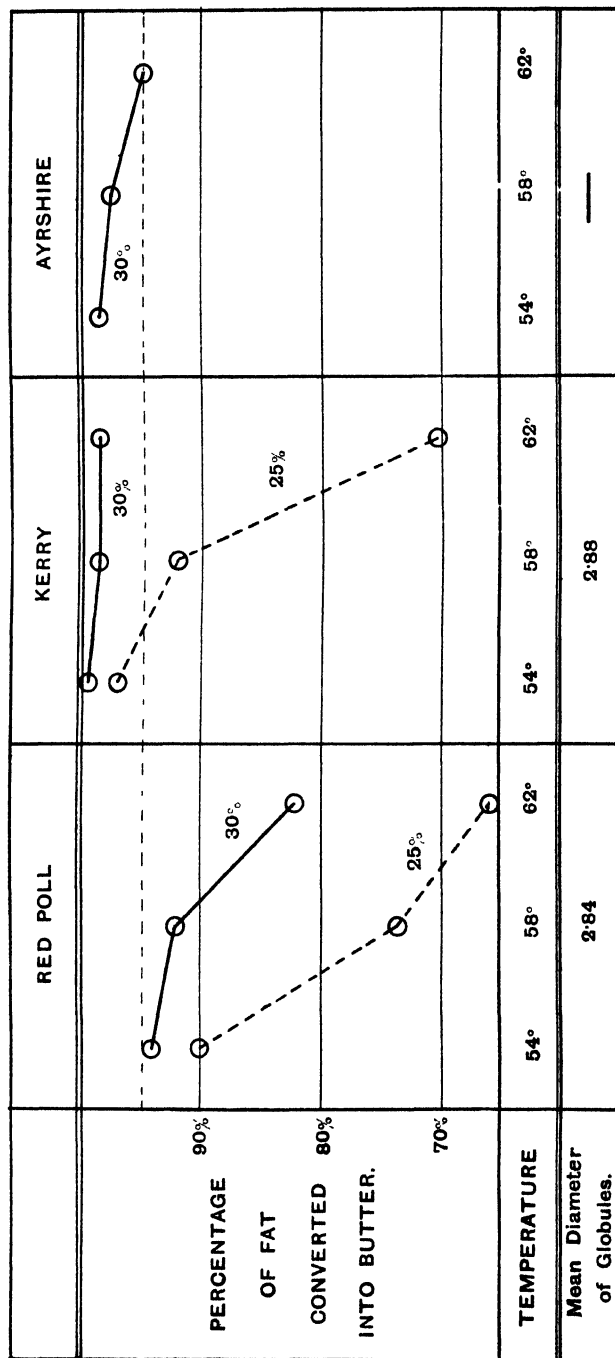


Fig. 1. Curves showing effects of different temperatures on the percentage conversion of milk fat into butter.

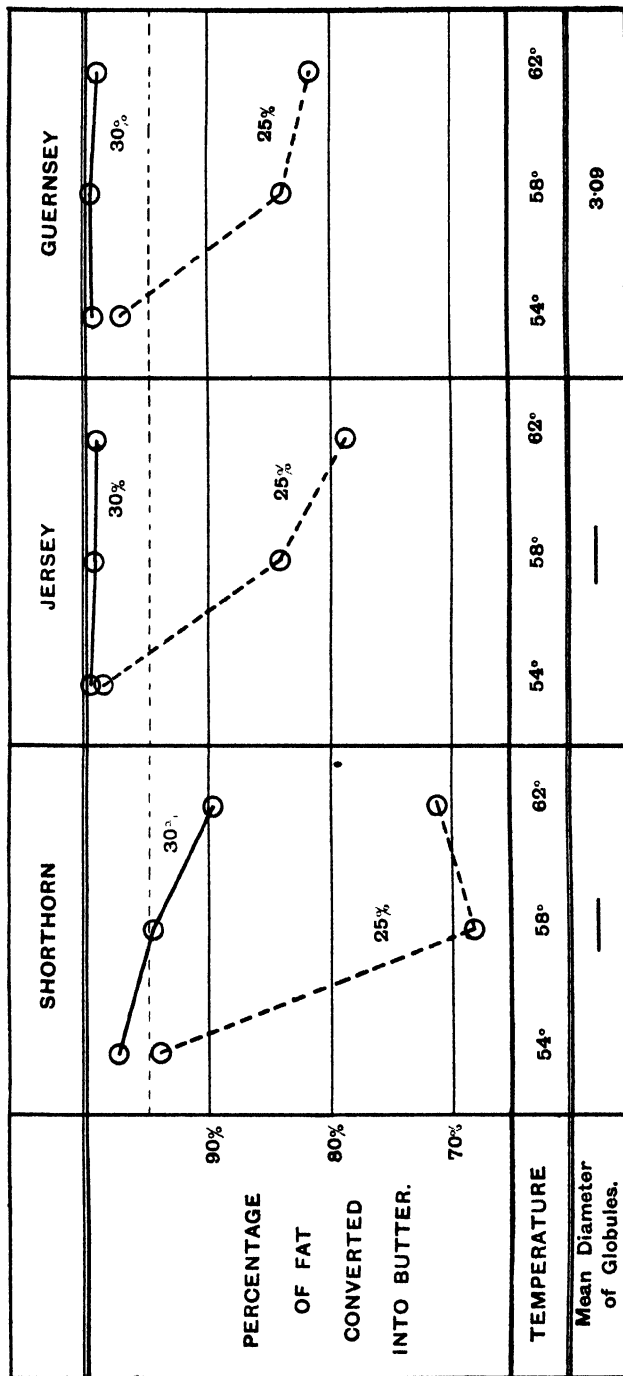


Fig. 2. Curves showing effects of different temperatures on the percentage conversion of milk fat into butter.

For reference, the mean size of the fat globules contained in the milks from which the creams were obtained is given below three of the curves.<sup>1</sup> No correlation could be determined between the percentage conversion and the size of globule.

That the size of globules has an effect on the percentage conversion, however, is shown by Woll (Digestion Expts., *Seventh Ann. Report, Agric. Expt. Stat., Wisconsin*, 1890, 238; also *Agricultural Science*, vi, 1892, 545), who concluded that "large size in the fat globules facilitates both the creaming and the churning of the milk; but the size of the globule is only one of the factors influencing completeness of creaming in churning processes." By mixing milks containing globules of different sizes "the creaming efficiency does not seem to be materially affected."

#### THE LIMITING-SURFACE OF THE GLOBULES.

In problems connected with churnability, a knowledge as to the presence or absence of a covering to the globules is important.

A very brief account of the different views put forward may be of interest. The various opinions may be classified in three categories. There are those who hold that there is an actual skin, membrane or pellicle round the globule. There are those who consider that there is nothing more than a "sliminess" round the globules; a condition which appears to us to be described best as a *mucilaginous aggregation*. Finally, others deny the existence of any covering whatever.

The membrane theory was first put forward by Raspail (*Schmidt's Jahrbuch*, xxiv) and by Acherson (*Müller's Arch. f. Anat. u. Physiol.*, LIII, 1840), so that the pellicle is often termed Acherson's membrane, though Acherson himself suggested the term *haptogen membrane*. Voltz (*Pflüger's Arch. f. Physiol.*, cii, 1904, 373) carried out a lengthy and detailed research, and concluded that "the fat globules of milk possess pellicles of solid substances, probably actually *solid membranes*."

Of the second view, perhaps the best exposition is that of Storch ("On the structure of the 'Fat Globules' in Cow's Milk," *Analyst*, xxii, 1897, 197—211), who isolated an albuminous substance which he claimed was the actual membrane. He expressly states, however, that "by the term membrane, is not meant a solid film or skin, but merely a layer, a semi-fluid, viscous envelope,"—evidently a "sliminess" round the globules. The use of the terms "membrane," "skin," "envelope," etc.,

<sup>1</sup> The other figures cannot be given.

appears to be unfortunate for the description of such a condition; it causes a misconception, as is evident from some of the criticisms of Storch's work. For this reason, together with one to be mentioned later, we propose the term *mucilaginous aggregation*.

With regard to the substance isolated by Storch, Voltz (*Pflüger's Arch. f. Physiol.*, CII, 1904, 373) more recently has obtained a substance which surrounded the globules even after they had been very thoroughly washed, but he found that there were extraordinary variations in the composition, according to the conditions of the experiment. Both these workers were able to stain the substance round the globule, but each drew different conclusions.

In our opinion, the divergent results obtained and the views held by the different workers are all to be reconciled on the theory that *constituents of the milk serum are adsorbed to the surface of the globule*.

On this theory, milk would not differ essentially from many artificial emulsions. It is generally accepted that milk differs from an ordinary emulsion in that the fat is not extractable with ether. If sufficient ether is used, however, the fat can be removed completely. Further, a number of artificial emulsions—*cyllin* in water, for example—behave exactly like milk in this respect, the oil being removed by ether only with great difficulty. In both cases the fat is comparatively easily extracted if the emulsifying agent is broken down. Moreover, it is possible to detect and to isolate the aggregated adsorbed emulsifying agent surrounding the globules in an artificial emulsion, so that it is only reasonable to conclude that similar conditions prevail in milk. In short, the difference between milk and an artificial emulsion appears to be one of degree only, and that difference is to be explained by the phenomenon of adsorption.

In this connection, the paper of Marshall (*Pharm. Journ. and Pharmacist*, Feb. 27th, 1909) and that of Ramsden ("Observations on Surface Membranes, Bubbles, Emulsions, and Mechanical Coagulation," *Chem. News*, LXXXVIII, 1903, 49—51; also *Proc. Royal Soc.*, LXXII, 1904, 458) are of considerable interest.

An actual solid membrane is not incompatible with the theory, as is clearly shown by Ramsden (1903) when discussing this theory:—"An intense *special interface viscosity*...has been found with every pair of liquids capable of forming persistent emulsions, hitherto examined." And "...the presence of a pellicle at the interfaces between caseinogen solutions and pure butter fat...." Also "the existence of a proteid

‘Haptogen-membrane’ round the cream globules of milk cannot be any longer doubted.”

Storch’s conclusions are based upon the result of one experiment. Voltz made repeated experiments, and obtained substances which varied greatly in composition according to the conditions. An examination of his figures shows that it is what one would expect, according to the adsorption theory, but they have not been submitted to mathematical analysis, since the data are insufficient.

#### FEEDING EXPERIMENTS—1912.

Several observers have noted that the food has an effect upon the globules, but, unfortunately, their results are contradictory.

Sturtevant (*Ann. Report U.S.D.A.*, 1880; Abridged Account in *Journ. Royal Agric. Soc. Eng.*, XVIII, (2), 1882, 475—495) concluded that *brans* and *shorts* diminished the size of the globule, whereas corn-meal “influenced uniformity.” Woll (*Sixth Ann. Report, Agric. Expt. Stat., Wisconsin*, 1889, 122; also *Seventh ditto*, 1890, 246) stated that dry food decreased the number and increased the size of the globules. Corn gave the same result as dry food. Gutzeit (*Landwirtsch. Jahrbücher*, Berlin, XXIV, 1895, 539—668), who carried out an extremely large number of observations, came to the conclusion that it was *not* true that one food would increase the diameter and another diminish it; but that, with a sudden change of fodder, there was a temporary change in the size.

It should be observed that the differences found by Woll were small, and, according to our experience, are within the limits of experimental error. Moreover, they are expressed in “relative sizes,” and not in mean diameters, as we have done; nor in mean volumes, as Gutzeit has done; so that the figures appear, at first sight, to be more conclusive than one might otherwise suppose.

As the matter is of great importance in the present work, it was considered advisable to carry out further experiments, not with any idea of ascertaining the influence upon the quantity and quality of milk, but solely with respect to the globules.

It was decided to take three sets of cows; to one, to give an ordinary food such as would be given normally, having a proteid-carbohydrate ratio of about 1:6; to another set of cows to give food having an abnormally high, and to the third set a food having an abnormally low ratio. The object of these abnormal foods was to

accentuate any difference, and it was considered advisable to accentuate them as much as possible, because there is a considerable difference of opinion as to whether the size of the globules is actually altered. Not only that, but there is a natural diminution in size as the period of lactation advances, so that, in any case, a diminution in size must be expected with those cows fed on the normal food; an effect which it was desirable to exclude as far as possible.

The experiments were carried out in a dairy very near to the laboratory, where some forty cows are milked. The breed is not pure, but is chiefly Shorthorn. They were being stall-fed, so that one could know exactly the quantity of food they received. Six cows were taken and were divided into three lots of two each, one lot for each ration.

In addition, Mrs Dudley Cory-Wright, of Dorking, very kindly allowed us to carry out similar experiments with three of her cows, two Guernseys and one Jersey. These received the same dry rations, but they were turned out, and therefore had an unknown quantity of grass, so that it is impossible to give ratios.

Photographs of the milk and other data were taken before the experiments commenced; then rations were given according to Table IV. Samples of the morning and evening milk were taken every third day. After 22 days<sup>1</sup>, a measurement of the globules showed no alteration, and it was decided to modify the rations, so as to accentuate the ratios to an even greater extent.

Determinations were made every third day, as before, for 26 days, and then the food of the cows was changed over; so that those cows which had been having the food with a high ratio were given the food with the low ratio, and vice versa. The cows receiving the food with the normal ratio continued as before.

### FOOD.

A decision as to the most suitable rations was very difficult, since discrepant figures are given for the composition of each material. As the chief authority, the figures of Crowther were taken. The rations given were based chiefly upon suggestions of Dr Hedworth Foulkes, and are tabulated below.

As to the materials, the grass was ordinary meadow grass, containing no clover. The hay was similar. The maize meal was ordinary material

<sup>1</sup> Woll (*Sixth Ann. Report, Agric. Expt. Stat., Wisconsin*, 1889, 71) also fed his cows 3 or 4 weeks, before changing the fodder.



purchased in Watford. The soya cake and cotton cake were obtained from Messrs Bibby, who very kindly made a reduction in price for the experiments, and who have been very obliging in many details. All the cake supplied was prepared specially in one batch, so that there should be no variation.

The rations shown in column A in Table IV were given for 22 days; after that the rations were given as shown in column B, having the corresponding ratios. The final examination was made on the 38th day. The cows were milked at 4.0 a.m. and at 1.0 p.m.

TABLE IV.

RATIONS (per cow per day)	High Albuminoid Ratio		Medium Albuminoid Ratio		Low Albuminoid Ratio	
	A.	B.	A.	B.	A.	B.
	For 22 days	After 22 days	For 22 days	After 22 days	For 22 days	After 22 days
Meadow grass (no clover)	42 lbs.	42 lbs.	56 lbs.	56 lbs.	42 lbs.	42 lbs.
Hay .....	—	—	—	—	14 lbs.	14 lbs.
Soya cake .....	4 lbs.	6 lbs.	—	—	—	—
Cotton cake (undec.) .....	—	—	3 lbs.	5 lbs.	—	—
Maize meal .....	—	—	—	—	4 lbs.	6 lbs.
DIGESTIBLE FOOD RATIO*	1 : 2.75 : 0.24	1 : 2.2 : 0.22	1 : 5.2 : 0.33	1 : 4.4 : 0.33	1 : 8.9 : 0.36	1 : 8.9 : 0.38
Total solids .....	12 lbs.	13.75 lbs.	14 lbs.	15.5 lbs.	24 lbs.	25.75 lbs.
QUANTITIES OF CONSTITUENTS PER RATION						
Total albuminoids .....	3.0 lbs.	3.8 lbs.	2.3 lbs.	2.9 lbs.	3.1 lbs.	3.3 lbs.
„ carbohydrates.....	5.3 „	5.8 „	6.6 „	7.3 „	13.0 „	14.3 „
„ fat.....	0.6 „	7.4 „	0.6 „	0.7 „	0.8 „	1.0 „
Digestible albuminoids .....	2.0 „	2.7 „	1.3 „	1.6 „	1.5 „	1.6 „
„ carbohydrates.....	5.5 „	5.9 „	6.8 „	7.2 „	13.1 „	14.4 „
„ fat.....	0.5 „	0.6 „	0.4 „	0.5 „	0.5 „	0.6 „

\* These ratios are arranged :—albuminoid, carbohydrate, fat.

The method of mensuration of the globules has been given in a former paper (*Journ. Agric. Sci.*, IV, Pt. 2, 1911, 155—166) but a detailed description, together with an account of Babcock's method, appears in a subsequent portion of this issue<sup>1</sup>, p. 357.

<sup>1</sup> It would be well to emphasise, once more, the futility of attempting to judge either of the average size of the globules or of the fat content in a milk, by visual examination. It must not be supposed that a field full of small globules contains more fat than one with a small number of large globules; or vice versa; determination alone can decide this.

It should be pointed out that, in Babcock's method, it is not possible to check the results. In our method, however, this can be done as pointed out below; any discrepancy may be due to erroneous counting, to bad sampling, or to selection of a bad field for the photograph, and the count can be neglected as being erroneous.

The two methods of computation of the mean diameter are<sup>1</sup>:—

A. The globules can be counted and the size of each measured, so that the distribution of the fat in the different sizes of globules can be determined. From the numbers so obtained curves can be drawn which show graphically the distribution of the fat.

B. Where the mean diameter alone is required, the number of globules in a known area is counted; the depth of the cell is known, so that the volume of milk in the area measured is known also; the percentage of fat, determined by analysis<sup>2</sup>, is reduced to give the volume of fat in the volume measured; this fat is distributed in the known number of globules, so that the mean diameter of the globules can be calculated. This is the method by which the mean diameters have been calculated for Tables VI—XIII and Figs. 3—6.

For some of the milks the mean diameters have been calculated also by method A, and the figures thus obtained are bracketed in Tables VI—X. The agreement or disagreement of these two figures is a measure of correctness of the determination, and an asterisk is placed against those in which there is an appreciable discrepancy.

An excellent instance of the value of this dual method of calculation is afforded by the following. The milk of Cow 10 on August 18, p.m., gave an analytical figure of 7.32 for the percentage of fat. This abnormally high figure appeared to be due to an error in analysis; but, using this percentage, the mean diameter was 3.41; whereas by the first method, not using the analytical figure, the mean diameter was 3.32. Such good agreement shows that the analytical figure was substantially correct.

<sup>1</sup> In our work published in 1911, p. 163, the centres of gravity of the curves A were given as representing the *mean* diameters. These are to be calculated from the number of globules in the area counted, and the percentage of fat. This will be rectified in due course. The use of the word "mean" was incorrect, and should have read "average." The distinction between these two terms, and their significance, is considered in detail in the account of the mensuration of the globules, p. 372.

<sup>2</sup> An important, and a very disconcerting source of error was experienced. The fat was determined in a centrifugal apparatus, using the amyl alcohol and sulphuric acid method. The milks, at first, showed great discrepancies. The tubes were then standardised, using a milk checked by the Adams method, and the following correction factors were found necessary:—1.03, 1.23, 1.18, 1.06, 1.10, 1.04.

It might be pointed out that the second method is, practically speaking, a modification of Babcock's original method, but the volume measured is much larger—about 10 times.

The results obtained from these feeding experiments have been tabulated, and, in order that they may be seen at a glance, curves have been drawn, as given below, pp. 350, 351.

Before discussing these tables, it should be pointed out that the number of cows was very small. But, although the calculations have been considerably simplified, owing to the adoption of the photographic method, yet the work is very laborious and all our available time was occupied. Moreover, the cost of the work was considerable, and would have been excessive had more cows been taken.

TABLE V.

Cow 7. Calved "lately" (? April).

Date	Morning				Afternoon			
	Average daily yield of milk	Fat	No. of globules	Mean diameter	Average daily yield of milk	Fat	No. of globules	Mean diameter
	lbs.	%		$\mu$	lbs.	%		$\mu$
22. 7. 12	22.5	3.33	1677	2.94* (3.94)	13.0	4.22	1397	3.38* (3.76)
25. 7. 12	23.25	3.0	—	—	16.25	3.77	—	—
28. 7. 12	22.4	2.83	—	—	15.4	3.44	—	—
31. 7. 12	20.5	3.22	1296	3.16 (3.12)	14.3	4.22	1740	3.16
3. 8. 12	22.3	3.39	1710	2.93	15.2	3.51	1609	3.03
6. 8. 12	22.3	3.07	927	3.48	14.7	3.84	1140	3.50
9. 8. 12	22.7	—	—	—	15.5	—	—	—
12. 8. 12	23.5	2.76	1019	3.25	14.7	3.90	1417	3.27
Food modified mid-day Aug. 13								
15. 8. 12	23.4	2.67	1280	3.01	16.3	3.84	1335	3.32
18. 8. 12	24.25	2.94	1527	2.91 (2.93)	16.75	3.72	1709	3.03 (3.24)
Food changed Aug. 18								
30. 8. 12	22.1	3.12	1573	2.94	14.7	3.48	1627	3.01

TABLE VI.

Cow 8. Calved "some time ago" (? March).

Date	Morning				Afternoon			
	Average daily yield of milk	Fat	No. of globules	Mean diameter	Average daily yield of milk	Fat	No. of globules	Mean diameter
22. 7. 12	lbs. 16.5	% 2.41	403	$\mu$ 4.58* (5.48)	lbs. 9.5	% 3.99	430	$\mu$ 4.91* (5.58)
25. 7. 12	17.7	2.52	—	—	10.1	5.29	—	—
28. 7. 12	17.2	2.50	—	—	8.8	4.83	—	—
31. 7. 12	16.7	2.44	258	4.92 (5.11)	9.1	5.16	509	5.05
3. 8. 12	17.7	2.78	363	4.60	9.3	4.52	578	4.63
6. 8. 12	16.7	2.71	297	4.87	9.25	4.44	328	5.57 (5.72)
9. 8. 12	16.3	—	—	—	9.1	—	—	—
12. 8. 12	16.3	2.94	523	4.15	8.25	3.84	494	4.62
Food modified mid-day Aug. 13								
15. 8. 12	16.8	2.46	288	4.77	9.3	4.14	399	5.09
18. 8. 12	17.75	1.98	314	4.32* (4.91)	10.3	5.16	721	4.50 (4.20)
Food changed Aug. 18								
30. 8. 12	16.6	2.52	341	4.55	9.4	4.56	677	4.41

TABLE VII.

Cow 9. Calved "considerable time ago" (? Nov. or Dec.).

Date	Morning				Afternoon			
	Average daily yield of milk	Fat	No. of globules	Mean diameter	Average daily yield of milk	Fat	No. of globules	Mean diameter
22. 7. 12	lbs. 12.0	% 2.61	1481	$\mu$ 2.82 (3.08)	lbs. 7.0	% 3.66	1729	$\mu$ 3.00 (3.19)
25. 7. 12	13.5	2.89	—	—	6.5	3.92	—	—
28. 7. 12	12.25	2.83	—	—	6.0	4.77	—	—
31. 7. 12	11.0	3.11	1940	2.74 (2.98)	5.8	4.61	2133	3.02
3. 8. 12	13.1	2.94	1488	2.93	6.6	4.26	1727	3.15
6. 8. 12	12.3	3.00	1162	3.20	5.5	4.03	1353	3.36
9. 8. 12	11.7	—	—	—	6.2	—	—	—
12. 8. 12	10.8	3.36	2029	2.76	5.5	5.04	1384	3.59
Food modified mid-day Aug. 13								
15. 8. 12	10.5	2.94	1730	2.78	5.4	4.32	1790	3.13
18. 8. 12	11.9	2.82	1578	2.83 (2.90)	6.0	4.38	2273	2.91 (3.17)
Food changed Aug. 31								
30. 8. 12	11.7	4.08	1343	3.38	6.1	4.68	1461	3.44

*Fat Globules in Milk*

TABLE VIII.

Cow 10. Calved "considerable time ago" (? March).

Date	Morning				Afternoon			
	Average daily yield of milk	Fat	No. of globules	Mean diameter	Average daily yield of milk	Fat	No. of globules	Mean diameter
22. 7. 12	lbs. 12.5	% 4.02	1616	$\mu$ 3.16* (1.49)	lbs. 7.25	% 5.50	1943	$\mu$ 3.30 (3.10)
25. 7. 12	13.9	3.66	1993	—	6.75	5.00	—	—
28. 7. 12	13.2	4.16	—	—	5.9	5.11	—	—
31. 7. 12	11.8	3.55	2061	2.38 (2.68)	5.9	4.44	2312	2.90
3. 8. 12	12.8	4.38	2504	2.81	5.8	5.21	2489	2.98
6. 8. 12	11.1	4.20	2086	2.95	5.9	4.56	2221	3.30
9. 8. 12	12.2	—	—	—	5.5	—	—	—
12. 8. 12	10.9	3.96	2220	2.83	5.3	5.64	1812	3.41
Food modified mid-day Aug. 13								
15. 8. 12	10.8	4.44	1612	3.27	5.1	6.24	1592	3.68
18. 8. 12	8.6	5.22	2411	3.02 (2.79)	4.3	7.32	2346	3.41 (3.32)
Food changed Aug. 18								
30. 8. 12	9.4	4.32	2538	2.79	4.9	5.52	2845	2.91

TABLE IX.

Cow 11. Calved "some time ago" (? Nov. or Dec.).

Date	Morning				Afternoon			
	Average daily yield of milk	Fat	No. of globules	Mean diameter	Average daily yield of milk	Fat	No. of globules	Mean diameter
22. 7. 12	lbs. 8.75	% 3.32	1427	$\mu$ 3.06 (3.23)	lbs. 5.25	% 3.55	1538	$\mu$ 3.09 (3.22)
25. 7. 12	10.9	3.00	—	—	5.6	3.66	—	—
28. 7. 12	10.5	3.93	—	—	5.2	3.89	—	—
31. 7. 12	9.75	3.61	1552	3.10* (3.61)	4.75	4.50	1665	3.25
3. 8. 12	10.9	3.11	1704	2.85	5.25	3.51	1852	2.89
6. 8. 12	10.75	2.83	1192	3.13	5.2	4.08	1799	2.44
9. 8. 12	10.1	—	—	—	5.4	—	—	—
12. 8. 12	10.6	2.94	1736	2.78	5.25	3.78	1516	3.16
Food modified mid-day Aug. 13								
15. 8. 12	10.75	2.88	1668	2.80	5.7	3.60	1644	3.08
18. 8. 12	11.3	2.40	1396	2.59 (2.53)	5.6	4.04	2261	2.88 (2.91)
Food changed Aug. 18								
30. 8. 12	9.6	3.69	1793	3.04	5.0	3.96	2019	2.92

TABLE X.

Cow 12. Calved "lately" (? April).

Date	Morning				Afternoon			
	Average daily yield of milk	Fat	No. of globules	Mean diameter	Average daily yield of milk	Fat	No. of globules	Mean diameter
22. 7. 12	lbs. 12·0	% 3·44	300	$\mu$ 5·27 (5·24)	lbs. 8·0	% 4·88	544	$\mu$ 4·85* (5·88)
25. 7. 12	13·5	3·89	—	—	8·25	4·72	—	—
28. 7. 12	14·0	3·11	—	—	8·0	4·99	—	—
31. 7. 12	11·2	3·16	300	4·36* (5·41)	8·25	4·77	608	4·64
3. 8. 12	15·1	3·62	339	5·14	8·4	5·49	688	4·66
6. 8. 12	14·7	3·00	237	5·44	7·0	5·52	530	5·09
9. 8. 12	13·5	—	—	—	7·5	—	—	—
12. 8. 12	13·7	2·88	229	5·43	7·6	5·16	494	5·10
Food modified mid-day Aug. 13								
15. 8. 12	13·3	2·70	196	5·59	8·2	4·56	516	4·82
18. 8. 12	13·25	3·66	527	4·46 (4·92)	7·1	4·80	—	—
Food changed Aug. 18								
30. 8. 12	12·25	3·60	416	4·80	6·5	6·36	605	5·12

The tables include determinations made upon the morning and the evening milk; for the curves, however, an average of these two daily measurements was taken. The "average yield of milk" represents the daily average for the three-day period. The fat was estimated on the centrifuge. The number of globules was determined, as already mentioned. The mean diameters were worked out from the number of globules and the percentage of fat—method B. Those figures for the mean diameter given in brackets were calculated from the actual measurements of the globules—method A. On August 13 (mid-day) the food was slightly modified; the rations were changed over on August 18, as shown.

One of the most noticeable features of the tables is that the numbers for each cow are very irregular, whether considering the fat, the number of globules, or the diameter. This is probably due to the examination of too small a volume of milk.

With regard to the curves, which have been plotted from these figures, in order to reduce any individual peculiarity of the cows, an average given by the two cows of each set has been plotted in the upper of the three curves (a). This curve, of course, does not give any absolute value, but it does show the variations during the period of the experiments.

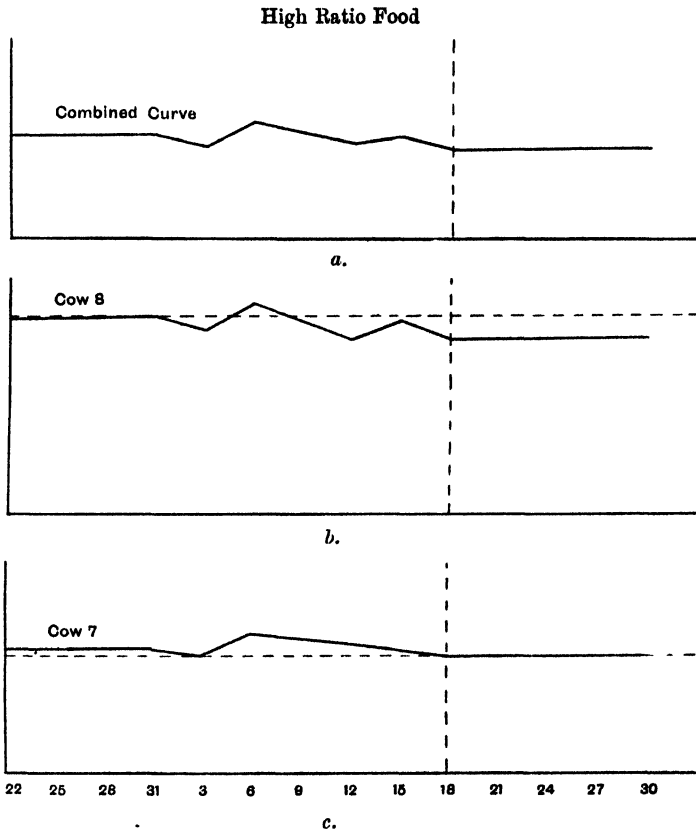


Fig. 3. Curves showing variation in mean size of fat globules during feeding experiments. The perpendicular dotted line indicates the point at which the High Ratio Food was changed to Low Ratio Food. Mean size of globules plotted as ordinates; dates as abscissae.

One would expect that the globules would diminish in size in any case, owing to the advancement of the period of lactation; but the curve is so irregular that one cannot observe a diminution in size, even due to this factor. Equally irregular variations have been experienced by others, as will be seen by an examination of Gutzeit's figures.

The only conclusion which can be drawn from these experiments is that the food has little or no influence on the size of the globules, if the "ratio" is taken as the criterion.

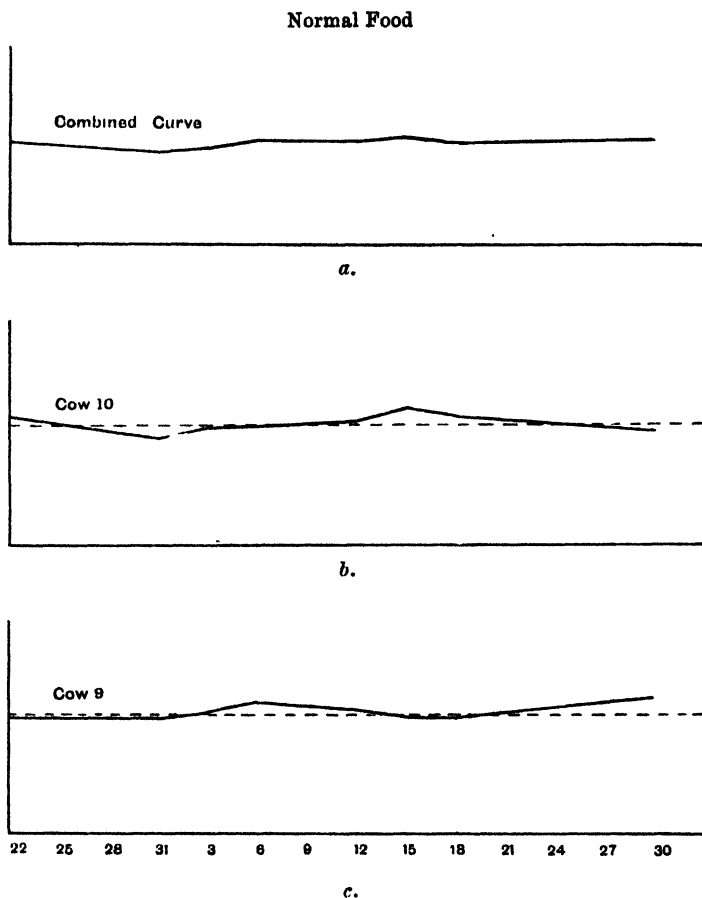


Fig. 4. Curves showing variation in mean size of fat globules during feeding experiments (control). Mean size of globules plotted as ordinates; dates as abscissae.

It is to be noted, however, that Woll (Digestion Expts., *Seventh Ann. Report, Agric. Expt. Stat., Wisconsin*, 1890, 238; also *Agricultural Science*, VI, 1892, 545) stated that it was *dry* food which gave the larger globules, but no information is given as to the *food ratio*. In our case, all the cows received grass, but the proportions were greater for those which had the food with low ratio; moreover, these cows had no cake, but maize meal and hay in place of it.



A plain food of grass only in the one case, and of dry foods only in the other, was not given in this series of experiments, because it was asserted by the herdsman that the cows would "go off their milk," owing to the character of their previous feed.

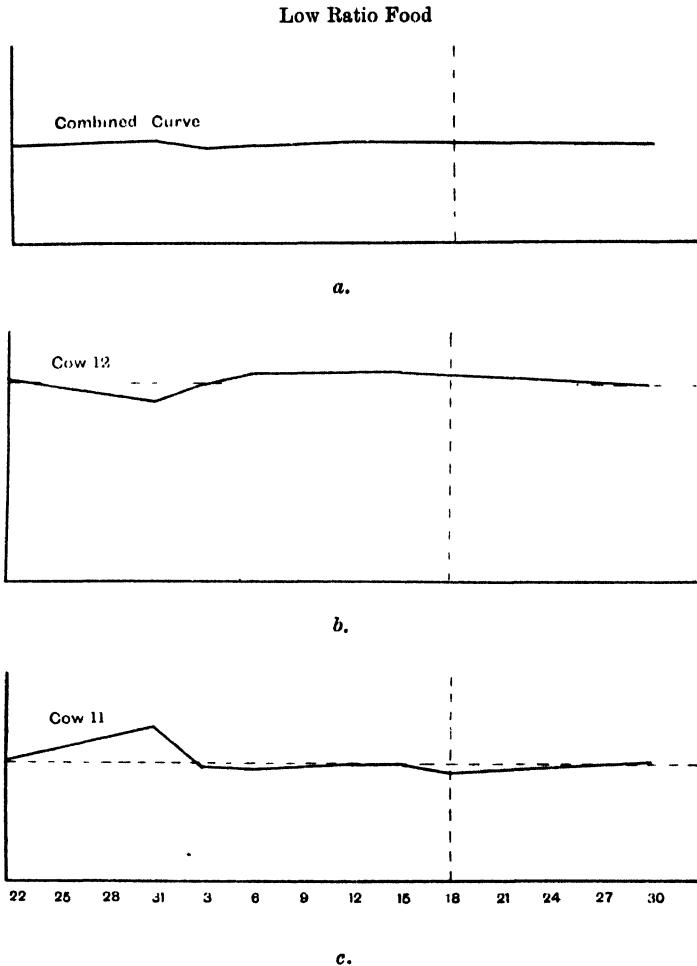


Fig. 5. Curves showing variation in mean size of fat globules during feeding experiments. The perpendicular dotted line indicates the point at which the Low Ratio Food was changed to High Ratio Food. Mean size of globules plotted as ordinates; dates as abscissae.

It is proposed to repeat the experiments, giving some cows a "dry" food and others grass only. In these experiments, it is suggested that observations should be made on the effect of the foods

upon the character of the serum and upon the churnability; for, in connection with previous experiments, it has been noted that there seems to be some relationship between the time of churning, the colour and quality of the butter, and the size of the globules. It is proposed also to make more determinations on each sample, taking them at longer intervals. Referring to the Tables V—X once more, it is to be noticed that there is a considerable difference in the mean diameter of the globules of the morning and evening milk on the same day; generally, those of the afternoon are the larger.

It may be observed, also, that in a number of the samples the percentage of fat is below the Government standard, and that in some it is abnormally high—a fact which can scarcely be due to sampling, as this was done within a short time of milking.

The number of globules in the milk of the same cow, at different periods, varies very considerably; in one case, a variation from 328 to 721 globules per unit volume in twelve days.

The results obtained from the cows at Dorking have been tabulated and curves drawn from them (Tables XI—XIII and Fig. 6). Here, again, it would appear that the feeding has had no effect on the globules. In this case, one may fairly presume that the cows had a very much larger quantity of *wet* food, yet no diminution in size of the globules is to be observed. With these cows, there is a much greater regularity in the size of the globules.

TABLE XI.

Cow 1. Jersey.

Date	Morning				Afternoon			
	Average daily yield of milk	Fat	No. of globules	Mean diameter	Average daily yield of milk	Fat	No. of globules	Mean diameter
	lbs.	%		$\mu$	lbs.	%		$\mu$
Aug. 3	18.3	3.30	2167	2.68	12.0	4.98	3130	2.72
„ 6	17.7	—	—	—	12.4	4.68	3467	2.58
„ 9	17.75	—	—	—	11.5	4.62	—	—
„ 12	18.0	3.72	2633	2.62	11.5	4.74	3085	2.69
„ 15	17.1	3.96	1859	3.00	10.5	5.10	2672	2.89
„ 18	16.8	3.96	2397	2.76	11.1	4.92	2793	2.82

*Fat Globules in Milk*

TABLE XII.

Cow 2. Guernsey.

Date	Morning				Afternoon			
	Average daily yield of milk	Fat	No. of globules	Mean diameter	Average daily yield of milk	Fat	No. of globules	Mean diameter
Aug. 3	lbs. 15.9	% 3.90	1526	$\mu$ 3.19	lbs. 9.75	% 5.04	1540	$\mu$ 3.46
„ 6	15.9	4.38	1853	3.11	10.1	4.14	2117	2.92
„ 9	16.1	—	—	—	9.9	5.22	—	—
„ 12	16.0	3.96	1632	3.14	9.7	4.50	1685	3.24
„ 15	15.75	—	—	—	8.9	4.74	1420	3.49
„ 18	15.4	4.32	1487	3.28	9.5	4.92	1841	3.24

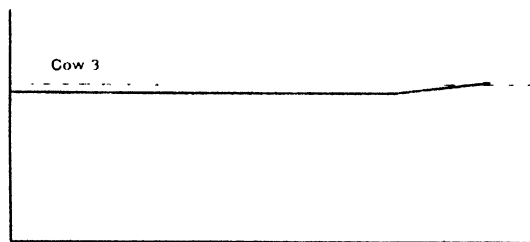
TABLE XIII.

Cow 3. Guernsey.

Date	Morning				Afternoon			
	Average daily yield of milk	Fat	No. of globules	Mean diameter	Average daily yield of milk	Fat	No. of globules	Mean diameter
Aug. 3	lbs. 16.75	% 4.80	1099	$\mu$ 3.81	lbs. 10.9	% 5.76	1347	$\mu$ 3.79
„ 6	16.8	3.96	1085	3.59	11.25	—	—	—
„ 9	16.6	4.62	—	—	9.9	5.10	—	—
„ 12	15.0	5.40	1415	3.65	10.5	7.08	1557	3.87
„ 15	15.2	4.68	1237	3.64	9.5	6.00	1345	3.84
„ 18	14.8	4.80	862	4.14	9.7	6.06	1097	4.13

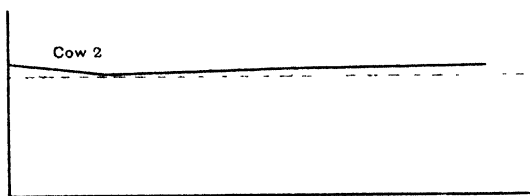
There is one point which appears to be of considerable economic interest. It is generally considered that Jerseys and Guernseys give milks with large globules, which cream well, whereas Shorthorns occupy a medium position. In the work of this year, the mean sizes of the globules are remarkable; the Jerseys and Guernseys are in agreement with the usual values, whereas those of the impure

Shorthorn are not only considerably larger than those of the Jerseys, but also they are larger than any mean sizes which have been seen by us, or recorded by Gutzeit, for any breed. Woll (*Eleventh Ann. Report, Agric. Expt. Stat., Wisconsin, 1894, 230*), however, records a Shorthorn milk having a mean size globule of  $7.5\mu$ , and gives other measurements of the same order as ours.



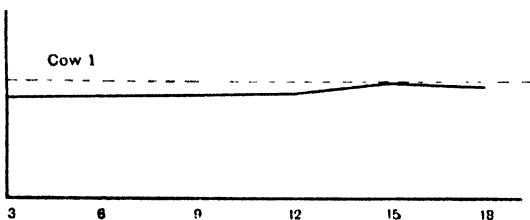
Low Ratio Food

1.



Normal Food

2.



High Ratio Food

3.

Fig. 6. Curves showing variation in mean size of fat globules during feeding experiments at Dorking. Mean size of globules plotted as ordinates; dates as abscissae.

These high values can scarcely be due to errors in counting, as they occur frequently. Neither can it be due to sampling, which might be the case if the milk had been standing some time before sampling,

when the upper layer would contain a greater number of larger globules, and also if the sample had been taken from this upper layer. The fact that an abnormally high percentage of fat was not found, precludes this possibility; on the contrary, the largest mean size occurs in the morning milk of cow 12, when the percentage of fat is the lowest recorded for that milk (2.70%).

Not only is it our experience that Shorthorn milks sometimes have a mean globule considerably larger than those of an average Jersey milk, but Woll (*Eleventh Ann. Report, Agric. Expt. Stat., Wisconsin, 1894, 230*), making determinations upon milks from a whole herd (99 cows), obtained similar figures.

If the size of globule is an important factor in dairying—as many consider—the question arises as to whether the *strain* of the cow may not be of more importance than the actual *breed*. That is to say, though, generally speaking, Jerseys are superior to Shorthorns for dairy work, yet certain *strains* of Jerseys may be inferior to certain *strains* of Shorthorns, and so with other breeds.

The only other suggestion bearing on this point, which we can find, is that contained in a paper of Cederholm (*U.S.D.A. Expt. Stat. Record, XII, 1901, 482*). He estimated the fat in the several cows' milks, and then that in the milks of their daughters by different bulls. His results show that each bull almost always causes an increase or a decrease in the percentage of fat. He concludes therefore "that the bull exerts a decided influence for better or worse on the milk product of his progeny."

This raises the question—important in its practical aspect—as to whether more attention should not be given to this point in attempting to improve our dairy herds. It is considered in actual practice, but scarcely sufficiently in interpreting experimental results.

## THE ENUMERATION AND MEASUREMENT OF FAT GLOBULES IN MILK.

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IN investigating the influence of various factors on the character of milk, it is now recognised that it is insufficient (at least for many purposes) to make comparisons of the percentage of fat, but some regard must be paid to the distribution of the fat in the globules. The technique of such investigations is somewhat complicated, and, as it was impossible to include in our previous papers a full description of the many points involved, it has been thought desirable to give the working details of the method employed, and at the same time to compare it with the method employed by previous workers.

It is hoped that these details may prove of considerable assistance to subsequent workers on similar problems.

Broadly speaking, there are two general methods for the enumeration and measurement of fat globules in milk; in one, the milk is examined in a capillary tube; in the other, in a flat cell. Both these methods were originally devised for the counting of corpuscles in blood, and have been adopted, with slight modifications, for milk examinations.

The capillary tube method was introduced for milk examinations by Bouchut, but, as Babcock made many extensive investigations with it, the method is usually associated with his name.

Babcock's original paper (*Fourth Annual Report, N.Y. Expt. Station, Geneva*, 1886, pp. 226, 275) is not generally available. Gutzeit, who has employed Babcock's method in a very exhaustive investigation on milk globules, has published, with his results, a full description of the method (*Landwirtschaftliche Jahrbücher*, 1895, 545). Since this journal is not easily accessible in this country, the authors

have made a detailed abstract of Gutzeit's description of Babcock's method.

The present authors have employed the flat cell method, with certain modifications, as it appears to present distinct advantages over that of Babcock.

## I. BABCOCK'S METHOD OF INVESTIGATION.

### *Detailed Abstract from Gutzeit.*

In order to measure the mean diameter of fat globules by Babcock's method, the number of globules in a measured volume of milk is determined. For this purpose, glass capillaries, of about 0.1 mm. diameter and 2—3 cms. long, are employed, the diameter being as constant as possible over small lengths.

10 ccs. of milk are diluted in a graduated flask to 500 ccs. With rich milk, or towards the end of the period of lactation, 5 ccs. may be used.

The capillary tubes are filled by dipping the end into the well mixed diluted milk, and the ends of the tubes are then sealed by melting in a small flame. The tubes are placed side by side, on a microscope slide, and fixed at both ends with a drop of melted wax. The slide is allowed to remain for half an hour on the microscope stage, which must be perfectly horizontal; a drop of water (or glycerine) is placed on the tubes, which are then covered with the cover-slip. By using an objective of sufficient magnification, the fat globules can be clearly recognised and counted through the capillary wall.

If the inner transverse diameter of the capillary is read off in terms of the divisions of the ocular micrometer, the ocular scale being at right angles to the length of the tube, and the micrometer focussed on the two bounding lines, the volume in which the enumerated globules are suspended can easily be calculated.

As we shall see later, the two lines do not represent the actual limits of the capillary walls, though they bear a definite relationship to them; their relative position is not affected, if the convergency of the illuminating rays (aperture of the sub-stage condenser) is altered, as would be the case if it were an interference phenomenon. Figure 1 shows the appearance of such a tube under the microscope at a magnification of 400 diameters, with an image of the ocular micrometer superposed.

A tube, 100 micrometer divisions long and 100 divisions broad, is taken as a *unit* tube; and the number of globules which would occur in a volume of milk required to fill such a tube is calculated by multiplying by 100<sup>2</sup> the number of fat globules found in a capillary 100 divisions long, and dividing by the square of the diameter of the capillary, measured in micrometer divisions.

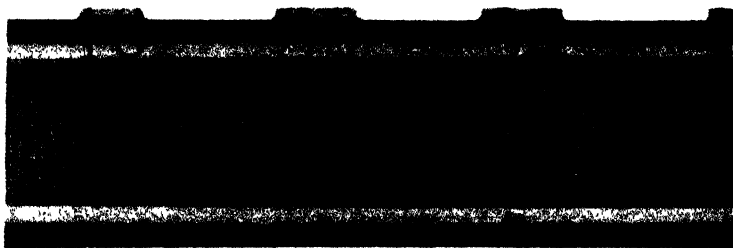


Fig. 1.

Thus, if  $a$  represents the number of enumerated globules and  $d$  the internal diameter of the tube, the number of globules occurring in a *unit* tube is given by the formula  $\frac{a \times 10,000}{d^2}$ , and, provided investigations are always carried out at the same dilution and magnification, the various values given by this formula may be directly compared.

In Gutzeit's experiments, one micrometer division corresponded to 0.0025 mm.; in Babcock's and Woll's work, to 0.0024 mm.

If  $d$  represents the internal diameter of the tube—measured in micrometer divisions—and the length of the tube always corresponds to 100 divisions, then the volume ( $J$ ) of the tube used for each measurement is given by the equation

$$J = \frac{d^2 \times \pi \times 100 \times (0.0025)^3}{4} \text{ mm.}^3$$

If, in this volume,  $a$  globules are counted, then 0.0001 mm.<sup>3</sup> of the diluted milk will contain

$$\frac{a \times 4 \times 0.0001}{d^2 \times \pi \times 100 \times (0.0025)^3} \text{ fat globules,}$$



and, since the milk has been diluted 50 times,  $0.0001 \text{ mm.}^3$  of milk will contain

$$\frac{a \times 4 \times 0.0001 \times 50}{d^2 \times \pi \times 100 \times (0.0025)^2} = \frac{a \times 4074}{d^2}$$

fat globules.  $0.0001 \text{ mm.}^3$  is taken as the "standard quantity," and the number of fat globules occurring in this quantity is designated by  $N$ . Thus

$$N = \frac{a \times 4074}{d^2}$$

In order to decrease the error due to the uneven distribution of the fat globules, 3 counts on each tube were made, thus 9 counts in all; 15 in some very careful estimations (Babcock and Woll made 5 counts on each of 3 tubes, but their micrometer scale was only half as long as Gutzeit's).

An example is given below.

*Montavon Cow "Rezia." 20th August.*

*Fat content 3.22 %/. Milk diluted 50 times.*

Capillary No.	I	II	III
$d =$	47	49	47
1st reading $a =$	124	150	177
2nd reading $a =$	190	170	153
3rd reading $a =$	183	194	166
Total	497	514	496
Mean	166	171	165

Using the formula previously given,  $\frac{a \times 10,000}{d^2}$ , these readings give 737 as the mean figure; that is to say, there are 737 fat globules contained in a *unit* tube. This number multiplied by the factor 0.4074, obtained above, gives 300 as the number of fat globules ( $N$ ) occurring in  $0.0001 \text{ mm.}^3$  of milk. Further, if the fat content of the milk ( $= f$ ) is known, there are the following relationships.

In  $0.0001 \text{ mm.}^3$  of milk there are  $\frac{0.0001 \times f}{100} \text{ mm.}^3$  of fat. This quantity of fat corresponds to the quantity in  $N$  globules. The volume

of the mean size globule is, therefore,  $\frac{0.0001f}{100N} = 0.000,001 \times \frac{f}{N} \text{ mm.}^3$   
 This expression represents Babcock's "relative size." In the example given  $f = 3.22\%$ ,  $N = 300$ , the relative size

$$= \frac{3.22}{300} \times 0.000,001 \text{ mm.}^3 = 0.000,000,0107 \text{ mm.}^3$$

As Babcock himself pointed out, this expression does not give absolute results, since the fat content is expressed in weight percentage, and no allowance for the specific gravity has been made. If the mean specific gravity of milk is taken as 1.0315, and that of butter fat as 0.94 at 15°, the volume of the mean size globule ( $Vd$ ) is obtained by multiplying the value for the relative size by  $\frac{1.0315}{0.94} = 1.1$ . This figure is used as a constant throughout this work.

In the above example  $Vd = 0.000,000,0107 \times 1.1 = 0.000,000,0118 \text{ mm.}^3$ . From the mean volume, the mean diameter of the globules can be calculated, but as all variations in the mean size are more evident when expressed as volumes, Gutzeit, like Babcock and Woll, compared the mean volumes, and only exceptionally calculated the mean diameters.

In order to be able to deal better with the small numbers involved, the micromillimetre ( $=\mu = 0.001 \text{ mm.}$ ) was taken as unit of length, and  $\mu^3$  as unit of volume.  $Vd$  in the above example  $= 11.8\mu^3$ , the "standard quantity" for the count of globules  $= 100,000\mu^3$ , the division of the micrometer scale  $= 2.5\mu$ , etc.

To obtain a clear image of the tubes, it is necessary to mount them in a liquid, as described; for this purpose Babcock and Woll used concentrated glycerine. The value for  $d$  varies according to the liquid used; the same tube for instance having an apparent diameter of 65 micrometer divisions in air, 56 in water, and 52 in glycerine. This phenomenon is due to the refraction of the light from the glass into the less dense liquid. Allowance can be made for it by dividing the diameter of the tube, read off on the micrometer, by the refractive index of the glass, of which the tube is composed, compared with that of the mounting liquid. If the mounting liquid, for instance, Canada Balsam, has the same refractive index as the glass, the measurements given by the microscope represent the real diameter of the tube. As a matter of fact, Gutzeit found it more convenient to mount the capillary tube in water, the index of refraction always being 1.333, and to calculate the value for  $d$ . For this purpose, the index of refraction

of the glass used was taken as identical with that of Canada Balsam, which was found, by means of an Abbe refractometer, to be 1.514; the refractive index of glycerine is 1.451. Therefore the factor required to convert  $d$  to an absolute value is  $\frac{1.333}{1.514} = 0.88$  if water is used, and  $\frac{1.451}{1.514} = 0.96$  if glycerine is used. These factors may be obtained without the use of a refractometer, by measuring the same tube in Canada Balsam and in water.

$$\begin{array}{lll} d \text{ in Canada Balsam} & = 50.3 & 50.3 \\ d \text{ in water} & = 56.0 & 56.0 \end{array} = .89.$$

If absolute values are required, the diameter of the capillary having been determined in water or glycerine,  $N$  (the number of fat globules) must be divided by the square of the corresponding factor (0.88 in the case of water and 0.96 in the case of glycerine), and  $Vd$  (the mean volume) multiplied by it.

For instance, in the example previously given,  $a$  having been determined in *water* (as is the case throughout this work), the absolute value of

$$Vd = 11.8 \times 0.89^2 = 9.51\mu^3.$$

It is obvious from the foregoing that, theoretically, Babcock's method, with the slight modifications introduced, is reliable, and should give absolute values. Woll's parallel estimations with it agree well, differing by only a few per cent. (*Agric. Science*, 1892, 445).

Considering the examples given above, it will be noticed that the readings in the same tube differ by about 30%; this, however, is not surprising, as, at a dilution of 50 or 100 times, a constant and equal distribution of the fat globules is not possible. The three figures which are obtained from the formula  $\frac{N \times 10,000}{d^2}$ , viz. 752, 712, 747, agree within 6%; in the worst cases this difference amounted to 10%. If we take two complete estimations with three capillaries and five readings on each, we find, for instance:

4th January, 1893. *Milk from Municipal Stall.*  $f = 4.51\%$ .

1st experiment  $N = 488$ ,  $Vd = 10.19\mu^3$ .

2nd experiment  $N = 501$ ,  $Vd = 9.90\mu^3$ .

Difference	13	$0.3\mu^3$ .
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The difference between the two values for  $N$  is 13 globules; that between the two values for  $Vd$ ,  $0.3\mu^3$ , or only about 3%. Repeated

estimations gave similar results. Better results were usually obtained with a smaller fat content and a higher value for  $Vd$ .

More accurate values for  $Vd$  may be obtained, if desired, by employing a greater magnification and a more accurate measurement of  $d$  and by an increased number of counts on more than three tubes. As, however, the estimation of the fat, by means of the centrifuge, is only accurate to about 3% (calculated on the fat) even when the greatest care is taken, there is not much point in obtaining a more accurate figure for  $d$ . An idea of the accuracy of the value for  $Vd$ , as given by this method, may be obtained by calculating the mean diameter  $d$  from the formula  $V = \frac{4}{3}\pi r^3$ .

Estimation 1.	$d = 2.69\mu$ .
„ 2	$d = 2.66\mu$ .
Difference	$0.03\mu$ .

Since it is impossible to measure beyond  $0.1\mu$ , this difference is of no account.

In this connection, it may be mentioned that  $Vd$  for the same cow, within one lactation period, may vary as much as 300% from day to day.

Theoretically, a slight error might be introduced in the factor 1.1, as only mean values for the specific gravity of milk and for butter fat have been taken. The specific gravity of the milk could be determined easily in each case; that of the butter fat at  $15^\circ\text{C}$ . not readily. If, however, experiments are carried out over the whole period of lactation, such variations would be eliminated in the mean  $Vd$ .

The computation of the two values  $N$  and  $Vd$  is simpler than it appears. If, instead of reducing the value of  $d$ , measured in water, to an absolute figure, we divide the final factor by  $0.89^3$ , we have

$$N = \frac{a}{0.89^3 \times d^3} \times 0.4074 = \frac{a \times 0.5053}{d^3}.$$

Further, when the same number of readings are made with each of the three tubes, the divisions are saved, if the final factor is divided by  $3 \times 3 (= 9)$  for three readings,  $3 \times 5 (= 15)$  for five readings, and so on.

The following table has been constructed to indicate the constant, dependent upon the dilution of the milk, the number of tubes and the number of readings on each tube required in the foregoing equation.

No. of readings for each tube	Dilution	No. of tubes	$F$	$\log F$
3	1:50	2	·8422	·9254
	1:100	2	·1684	·2263
	1:50	3	·5614	·7493
	1:100	3	·1123	·0504
5	1:50	2	·5053	·7035
	1:100	2	·1010	·0048
	1:50	3	·3374	·3374
	1:100	3	·6748	·8291

In addition, to obviate the three divisions by  $d^2$ , the following table was used, in which, for each value of  $d$ , measured in micrometer divisions, the tubes being mounted in water, the value for  $10 - 2 \log d$  can be read off.

$d$	$10 - 2 \log d$	$d$	$10 - 2 \log d$	$d$	$10 - 2 \log d$	$d$	$10 - 2 \log d$	$d$	$10 - 2 \log d$
30	0458	40	7959	50	6020	60	4437	70	3098
31	0172	41	7744	51	5849	61	4293	71	2974
32	9897	42	7535	52	5670	62	4157	72	2853
33	9630	43	7332	53	5515	63	4013	73	2743
34	9371	44	7132	54	5352	64	3876	74	2615
35	9112	45	6936	55	5192	65	3742	75	2499
36	8873	46	6746	56	5036	66	3609	76	2384
37	8635	47	6558	57	4884	67	3478	77	2271
38	8404	48	6375	58	4731	68	3349	78	2158
39	8179	49	6196	59	4583	69	3223	79	2048

*Translator's Note.* The values given in this table are not those of  $10 - 2 \log d$ , but merely the complements of the mantissae of  $2 \log d$ . The object of this procedure is to confine the use of logarithms to additions, eliminating all subtractions.

A calculation, using the example previously given, follows:—

“Rezia.” 20th August.  $f = 3.22$ . Milk diluted 50 times.  
3 counts on 3 tubes.

Capillary No.	I	II	III
$d =$	47	49	47
1st reading $a =$	124	150	177
2nd reading $a =$	190	170	153
3rd reading $a =$	183	194	166
Total	497	514	496

The corresponding value for  $10 - 2 \log d$  is added to the logarithm of each of these three totals:

log 497	6964	log 514	7110
$10 - 2 \log d$ (47)	6558	$10 - 2 \log d$ (49)	6196
log 225	<u>3522</u>	log 214	<u>3306</u>
	log 496		6868
	$10 - 2 \log d$ (47)		<u>6558</u>
	log 225		<u>3516</u>

The sum of these three figures is taken,  $225 + 214 + 225 = 664$ . To calculate  $N$  from this value, it is only necessary to multiply by the factor given in the table of constants above. Thus, for three readings on three tubes at a dilution of 1 : 50, the factor is 0.5614, the logarithm of which is 7493. To this is added the logarithm of 664,

log 5614	7493
log 664	<u>8222</u>
log 373	5715 = log $N$ .

For the calculation of  $Vd$ , the logarithm of  $f$  ( $= 3.22\%$ ) is added to that of 1.1 and the complement of the mantissa of log  $N$  is also added. Thus:

	log $f$	5079
	log 1.1	0414
Complement of the mantissa of log $N$ (5715)	4285	
	log 9.50	<u>9778</u> = log $Vd$ .

In addition to the determination of  $N$  and  $Vd$ , the percentage of the globules greater than  $2.5\mu$  and  $5\mu$  in diameter were calculated. For this purpose, a special count of the number of globules, exceeding one, and those exceeding two, divisions of the scale was made after each ordinary count.

Observations on the milk of "Rezia," 20th August, will again serve as an example:

	Tube No. I, $d = 47$			Tube No. II, $d = 49$			Tube No. III, $d = 47$		
	$a$	$> 2.5\mu$	$> 5\mu$	$a$	$> 2.5\mu$	$> 5\mu$	$a$	$> 2.5\mu$	$> 5\mu$
1st reading.....	124	55	4	150	43	6	177	48	4
2nd reading ..	190	65	5	170	60	4	153	55	5
3rd reading ..	183	63	4	194	87	6	166	74	8
Total .....	497	183	13	514	190	16	496	177	17

Total No.	$> 2.5\mu$	$> 5\mu$	
497	183	13	$550 \times 100$
514	190	16	$1507 = 86.5\% > 2.5\mu.$
496	177	17	$46 \times 100$
1507	550	46	$1507 = 3.0\% > 5\mu.$

As in counting the globules greater than two divisions we have also included those greater than one, the latter figure must be subtracted from the first to give the percentage required. Thus there are

33.5% greater than  $2.5\mu$ ,

3.0% „ 5μ.

After a little practice, the size of the individual globules can be estimated with a fair degree of accuracy, as shown by the following example.

The figures, employed to demonstrate the accuracy of  $Vd$ , are again used.

1st experiment  $N = 488$ ;  $Vd = 10.19\mu^3$ ;  $31.31\% > 2.5\mu$ ,  $1.90\% > 5\mu$ .

2nd experiment  $N = 501$ ;  $Vd = 9.9\mu^s$ ;  $31.30^\circ/\mu > 2.5\mu$ ,  $1.95^\circ/\mu > 5\mu$ .

Further differentiation between the size of globules is not possible by this method, as, with such great dilutions, there are insufficient globules within the field of the micrometer. If, however, such an estimation appears desirable, the whole of the tube, covered with the slip, may be enumerated.

## II. FLAT CELL METHOD.

In outline, the method consists in making a photomicrograph of the milk, contained in a Thoma-Zeiss Cell, the enumeration and measurement being carried out on a print.

### A. Photomicrography of the Milk.

The apparatus and method employed are as follows:—

*Apparatus.*

(i) A large *Zeiss Photomicrographic apparatus* is used. This consists of two benches, one supporting the entire optical system—the other the camera. For a full description and illustration of this apparatus, the reader is referred to Zeiss' Catalogue.

The microscope used was a No. 4 projection ocular. The extension of the camera was such as to give a magnification of 500 diameters.

The cell, containing the milk, must be kept in a horizontal position, in order to prevent the fat globules collecting together in one portion

of it. The microscope is therefore used in a vertical position and a reversing prism is employed to render the beam horizontal.

(ii) *Illumination.* This is obtained from a hand-fed arc (carbons at right angles), burning about 13 ampères.

(iii) *Exposure.* As the fat globules are in continual movement (Brownian), a short exposure is necessary. A Unicum Shutter is interposed in the beam of light, and adjusted to give an exposure of about 1/50 second. The plates used were Paget Extra Special Rapid (Speed about 450 H. and D.), and a normal metol-hydroquinone developer was employed.

(iv) *The Cell.* The Cell is a specially shallow Thoma blood counting cell, the depth being 0.015 mm. ( $15\mu$ ). The area of each square is 1/400 sq. mm.; so that the side of one square is 0.05 mm. An area of 16 such squares is photographed, so that, at a magnification of 500 diameters, the side of the large square used for counting is

$$0.05 \times 4 \times 500 = 100 \text{ mm. (or about 4 inches).}$$

Such a square is conveniently photographed on a half plate.

#### *Method.*

(i) *Adjustment of Magnification.* The clean empty cell is placed on the microscope stage and adjusted so that an image of the ruling of the squares is projected on to the ground glass screen of the camera which is then extended to give a magnification of exactly 500 diameters.

(ii) *Preparation of the Cell.* The glass slide and cover slip are both washed with soap and water (on a piece of cotton wool), thoroughly rinsed with water, and dried with a soft cloth.

The sample of milk is shaken sufficiently to ensure thorough mixing, but care must be taken not to churn it. A small portion is transferred, by means of a platinum loop, on to the centre of the cell, the cover slip is then lowered (by means of a needle) on to the slide and pressed gently, near the edges, till perfect contact is obtained. This is the case when Newton's rings can be seen at the surface of contact of the slide and cover slip. Some practice is necessary to judge the correct quantity of milk to be taken for the cell preparation, but, when the loop has once been adjusted, this is a very simple matter.

(iii) *Focussing.* In focussing the image on the ground glass screen, some difficulty is encountered, as, owing to the refraction of the light by the globules, no very definite outline can be obtained. Moreover, the globules float up to the underside of the cover-glass, so that the small globules are not in exactly the same plane as the large ones. With practice, however, very good results with sharp outlines can be obtained.



*B. Enumeration of the Fat Globules.*

The distribution of the fat in the various sized globules can be determined from the photographs in two ways:

(i) The *total number* of globules is determined in a definite area of the photograph—and therefore in a known volume of the milk. This figure, and the percentage of the fat as given by analysis, will give the “mean-diameter” of the globule.

(ii) The *diameter of each globule* in a definite area of the photograph is measured; and from this, without the aid of any analytical figure, the actual distribution of fat in the various sizes of globules can be computed.

The two methods are detailed below.

*Method 1.*

The photomicrographic print, obtained as already described, is ruled to correspond with 16 of the squares on the cell. Thus at 500 diameters magnification, the side of each small square will measure 25 mm., and the side of the square containing 16 of these will be 100 mm.

The *total number* of globules in each of the 16 small squares is then counted, each globule being marked off with a pencil as it is recorded; this ensures that each globule is counted once, and once only. The numbers for each of the 16 squares are then added, giving the total number for the whole area. Any globules cutting either of two adjacent sides of the large square (e.g. the top and left hand side) are included; whereas those cutting either of the two remaining sides are neglected.

The percentage of fat in the milk is determined by the centrifuge method.

Since all measurements made on the photograph relate to volumes, and since the analytical result is expressed as Wt/Wt percentage, a correction must be applied to correlate the two sets of figures.

Specific gravity of milk fat was taken to be 0.930.

„ „ milk was taken to be 1.034.

Then to convert Wt/Wt percentage to Vol/Vol percentage multiply by

$$\frac{1.034}{.930} = 1.1118,$$

$$[\log = 0.0460376].$$

*Volume of the Cell.*

16 squares are measured and the area of each square =  $\frac{1}{400}$  mm.<sup>2</sup>

The depth of the cell is 0.015 mm.

Therefore the volume measured is

$$\frac{16 \times 0.015}{400} \text{ mm.}^3 = 0.0006 \text{ mm.}^3 (= 600,000 \mu^3).$$

*Calculation of the "Mean Diameter."*

If  $d$  and  $v$  represent respectively the diameter and volume of a sphere, then

$$v = \frac{\pi d^3}{6} \text{ or } d = \sqrt[3]{\frac{6v}{\pi}}.$$

Let  $f = \%$  of fat (Wt/Wt) found by analysis, and  $n = \text{No. of globules in the measured volume}$ , then  $100 \mu^3$  of milk contain  $f \times 1.1118 \mu^3$  of fat.

Therefore  $600,000 \mu^3$  (the volume measured) contains

$$f \times 1.1118 \times 6000 \mu^3 \text{ of fat.}$$

Therefore 1 globule will contain  $\frac{1.1118 \times 6000}{n} \mu^3$  of fat. This represents the volume of the mean sized globule.

Therefore, if  $D = \text{diameter of the mean globule}$ , then

$$D = \sqrt[3]{\frac{6 \times f \times 1.1118 \times 6000}{n\pi}}.$$

In practice, logarithms are used for the calculation of  $D$ . An actual example will render the method of working quite clear.

*Example.*

A sample of milk contained 3.96% of fat, and the counted volume of the milk contained 1632 globules.

$$D = \sqrt[3]{\frac{6 \times 1.1118 \times 6000}{\pi} \times \frac{f}{n}} = \sqrt[3]{\frac{6 \times 1.1118 \times 6000}{\pi} \times \frac{3.96}{1632}}.$$

log 6	= 0.7781513	} →	log $\frac{6 \times 1.1118 \times 6000}{\pi}$	=	4.1051794
log 1.1118	= 0.0460267		add log 3.96 ( $f$ )	=	0.5976952
log 6000	= 3.7781513				4.7028746
	4.6023293		Subtract log 1632 ( $n$ )	=	3.2127202
log $\pi$	= 0.4971499		Divide by 3		3)1.4901544
	<u>4.1051794</u>			<u>0.4967181</u>	

$$= \log \text{ of } 3.1385.$$

$$\text{Hence } D = 3.14 \mu.$$

In calculating a series of such figures, the factor  $\frac{6 \times 1.1118 \times 6000}{\pi}$  remains constant and so, once obtained, the logarithm of this factor is simply added to that of  $f$ , the logarithm of  $n$  is subtracted and the result divided by 3 to obtain the cube root.

### *Method 2.*

In this method, not only is a determination made of the number of globules in the ruled area of the photograph, but the diameter of each globule is measured.

For this purpose, a transparency is made of a series of circles, to correspond with 1, 2, 3, ...  $\mu$  diameter. The magnification of the photographs was 500 diameters, so that the smallest circle was 0.5 mm. or  $500\mu$  diameter; the next 1.0 mm.; 1.5 mm., and so on; corresponding to 1, 2,  $3\mu \times 500$ . This transparency is superposed on the photograph and the diameter of one globule is measured; this is noted and the globule is crossed through with a pencil; another globule is measured, the diameter noted, the globule crossed through; and so on. In this manner, each globule is measured once and once only, and a complete record is obtained of the globules in the counted area. One small square ( $2.5 \times 2.5$  cms.) is enumerated before proceeding to another, the final result being obtained by the addition of the figures from the 16 squares.

This method of enumeration claims the attention of two workers, one making and calling out the measurements, the other recording them. It is quicker to select all the globules of one size first. After some practice, it is not found necessary to gauge every globule, but only the first few of each size. Since it is impracticable to differentiate between globules of less diameter than  $1\mu$ , all such are recorded as  $1\mu$  globules. Moreover, no fraction of  $\mu$  is taken into account, but the nearest whole figure is recorded.

From the figures thus obtained, three results may be calculated :

- (a) The mean diameter of the globules.
- (b) The actual distribution of the fat in the various sizes of globules.
- (c) The percentage of fat in the milk.

### *(a) Calculation of "Mean Diameter."*

The volume of fat contained in a globule of any definite diameter, multiplied by the number of such globules present, gives the volume of

fat contained by all the globules of that size. This operation is repeated for each size of globule in the milk. The sum of these figures gives the total volume of fat present in the measured volume of milk. If the volume, so obtained, be divided by the total number of globules, the volume of the mean sized globule results; from this, it is a simple matter to calculate the diameter of the mean sized globule. The following example shows the method of calculating the volume of the various sized globules ranging from  $1\mu$  to  $14\mu$  in diameter, and also of calculating the diameter of the mean globule.

Diam. of globules $\mu$	1	2	3	4	5	6	7	8	9	10	11	12	13	14
No. of globules	512	342	241	155	72	45	20	19	3	3	1	1	0	0
Total No.	1414													

$d$	$v$	$\log v$	$n$	$\log (v \times n)$	$v \times n$
1	$\frac{\pi}{6} \mu^3$	1.7189986	512	2.4282686	268
2	$\frac{8 \times \pi}{6} \mu^3$	0.6220886	342	3.1561147	1432
3	$\frac{27 \times \pi}{6} \mu^3$	1.1503624	241	3.5323794	3407
4	$\frac{64 \times \pi}{6} \mu^3$	1.5251786	155	3.7155103	5194
5	$\frac{125 \times \pi}{6} \mu^3$	1.8159086	72	3.6732411	4712
6	$\frac{216 \times \pi}{6} \mu^3$	2.0534524	45	3.7066649	5089
7	$\frac{343 \times \pi}{6} \mu^3$	2.2542927	20	3.5553227	3592
8	$\frac{512 \times \pi}{6} \mu^3$	2.4282686	19	3.7070222	5094
9	$\frac{729 \times \pi}{6} \mu^3$	2.5817261	3	3.0588474	1145
10	$\frac{1000 \times \pi}{6} \mu^3$	2.7189986	3	3.1961199	1571
11	$\frac{1331 \times \pi}{6} \mu^3$	2.8431767	1	2.8431767	697
12	$\frac{1728 \times \pi}{6} \mu^3$	2.9565423	1	2.9565423	905
13	$\frac{2197 \times \pi}{6} \mu^3$	3.060827	0	0	0
14	$\frac{2744 \times \pi}{6} \mu^3$	3.1573827	0	0	0

The total number is 1414.

The volume of fat in the volume of milk measured is  $33,106\mu^3$ .

The volume of the mean globule is  $\frac{33,106}{1414} = 23.41\mu^3$ .

The diameter of the mean globule ( $D$ ) =  $\sqrt[3]{\frac{6V}{\pi}} = \sqrt[3]{\frac{6 \times 23.41}{\pi}} = 3.55\mu$ .

The value for the mean diameter as given by these two methods should be in a fairly close agreement, and where such is not the case, a further examination of the milk can be made. It should be noted that, in Method 1, the percentage of fat determined analytically has been employed; in Method 2, no analytical figure is required, so that if the results, given by the two methods, are in agreement, there is little question of their accuracy.

(b) *Distribution of the Fat in the various sized Globules.*

Referring to the previous table, it will be seen that the figures under " $v \times n$ " are a measure of the quantity of fat present in any particular size of globule.

For instance, out of a total volume of fat of  $33,106\mu^3$ ,  $268\mu^3$  is present as  $1\mu$  diameter globules;  $1432\mu^3$  as  $2\mu$  diameter globules; and so on. It is a simple matter to transform these numbers into percentages, as has been done in the following table:

Diameter $\mu$	$v \times n$	% fat in various sized globules
1	268	0.81
2	1432	4.33
3	3407	10.29
4	5191	15.69
5	4712	14.23
6	5089	15.37
7	3592	10.85
8	5094	15.39
9	1145	3.46
10	1571	4.74
11	697	2.11
12	905	2.73
	33,106	100.00

From the last column, for instance, it will be seen that 15.37 % of the total fat is present in globules of  $6\mu$  diameter; 3.46 % in globules of  $9\mu$  diameter, and so on. A curve, connecting diameter and percentage of fat can be constructed from these figures. In figure 2, such a curve has been drawn (*B*), together with one showing the relationship between numbers of globules and diameters (*A*).

The centre of gravity of both curves is indicated. That of curve *B* represents the true "mean diameter"; that of curve *A*, the "average diameter," i.e. the value which would be arrived at from a purely visual examination, in which only two dimensions can be appreciated. The

large discrepancies between the values for these two centres of gravity is a forcible example of the futility of attempting to estimate the mean diameter by visual examination.

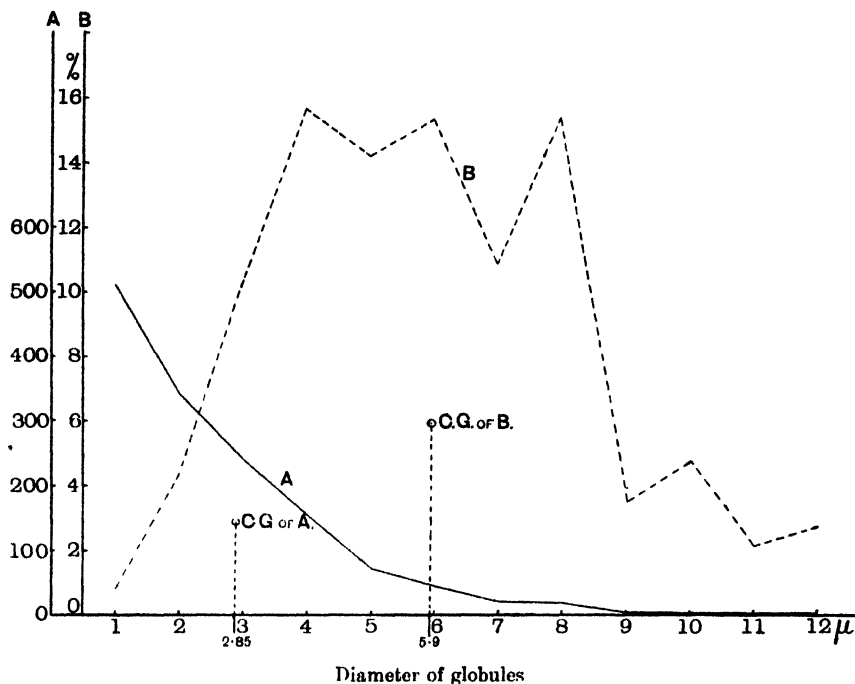


Fig. 2.

(c) *The Percentage of Fat in the Milk.*

The last column of the table shows that there are  $33,106\mu^3$  of fat in the volume of milk measured. This volume has already been shown to be  $600,000\mu^3$ . Hence the percentage of fat (Vol/Vol) in the particular sample of milk is

$$\frac{33,106}{6000} = 5.52 \% \text{ (Vol/Vol)}$$

$$= 4.96 \% \text{ (Wt/Wt)}.$$

The actual figure furnished by analysis was

$$4.80 \% \text{ fat (Wt/Wt)}.$$

These two figures should be identical, but if the small volume of milk measured (only  $0.0006 \text{ mm}^3$ ), and the difficulty of accurate measurement of the globules are considered, it will be seen that the agreement is as close as could be expected.

If the figure obtained by the above method differs considerably from that obtained by analysis, there is obviously an error in the determination; such an error may occur

- (i) in the determination of fat,
- (ii) in the selection of the sample for photography,
- (iii) in the measurement of the globules.

*Degree of accuracy of counting method.*

It was considered desirable to test the reliability of the method, and, to this end, three separate cell preparations and photographs were made on one sample of milk, and three more on another sample. The globules in each of the three photographs were counted, and the resulting figures were compared.

*Number of globules in counted area of photograph.*

Count	Sample A	Sample B
1	2105	302
2	2286	345
3	2288	397
Mean	2229	348

The difference between the numbers obtained from the triplicate photographs may be explained by

- (i) Errors in sampling.
- (ii) Slight difference in focussing the image.
- (iii) Errors in counting.
- (iv) Lack of "clearness" and density in the print.
- (v) Errors due to variation in the volume measured, owing to variation in temperature.

With care, the last three of these possible sources of error can practically be eliminated; the first is probably the most important, as only 0.0006 mm.<sup>3</sup> of milk is photographed.

### III. CONCLUSIONS.

Regarding the relative merits of the two methods, the authors have worked with both, and their preference for the cell method is based upon the following considerations.

In Babcock's method, measurements are made upon varying volumes of diluted milk, necessitating the use of a variable factor in the calculation of the mean volume.

In the cell method, a constant volume of undiluted milk is employed, and hence a constant factor is applied in all calculations. Moreover, the latter method dispenses with the use of an immersing liquid, and accordingly no correction for refraction is necessary.

Again, the volume of milk actually examined in the cell method is roughly ten times that measured in the capillary method.

Whilst several workers have made use of the cell method for milk globule enumerations, the application of photography to it is, as far as the authors are aware, novel. The innovation has several advantages. Foremost amongst these is the fact that permanent records are obtained, which may be enumerated at leisure. Since the film of milk photographed is so thin, no time is wasted waiting for the globules to rise (as is the case in the capillary method), and it is possible to make a very large number of exposures in a short period, the development, printing, counting, etc., being postponed, if necessary.

In Babcock's method, the effect of Brownian movement upon the smaller globules is liable to introduce two sources of error in counting. Firstly, the number of globules in the counted portion of the capillary is always changing, owing to the small globules at the limits of the measured length constantly leaving and re-entering the enumerated volume. Secondly, this constant oscillatory movement renders the counting of the small globules a matter of uncertainty. Since the calculation of Gutzeit's mean volumes ( $Vd$ ) rests upon the total number of globules counted, and the percentage of fat, it follows that the omission of a minute globule is as serious as that of a very large one.

Gutzeit himself points out that there is a difference of about 30%, in his parallel estimations, so that the above criticism is justified.

The use of a sufficiently short exposure in the photographic method completely obviates this difficulty. The parallel counts given on p. 374 differ by about 14% respectively—a much better agreement than Gutzeit obtained.

Another very distinct advantage afforded by the photographic method is, that it is possible, by making the double enumeration, as already described (p. 368), to check off the results, and so discard any doubtful values.

In spite of the fact that the method adopted by the authors involves the use of expensive apparatus, and the expenditure of a considerable amount of time, yet they are of the opinion that its advantages far outweigh these objections. It is true that Woll (*Agricultural Science*, 1892, p. 441) compared the capillary and cell methods and



concluded in favour of the former. He found that he always detected more globules, when using the capillary, than when using the cell. This is not surprising, as an examination of his technique reveals the fact that in the former case he was using a magnification of 950 diameters, whereas, in the latter, the magnification was only 400 diameters. Gutzeit appears to have accepted Woll's valuation of the reliability of the two methods without question.

A fair comparison of the two methods indicates the distinct superiority of the procedure adopted by the authors.

STUDIES IN MILK RECORDS: ON THE ACCURACY OF  
ESTIMATING A COW'S MILKING CAPABILITY BY HER  
FIRST LACTATION YIELD.

By WILLIAM GAVIN, B.A.

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A GOOD deal of uncertainty seems to exist among dairy farmers as to how far the first lactation yield may be taken as a guide to a cow's future milking career.

All know of course that it is by no means an infallible one, while some go so far as to ignore it altogether, and postpone "weeding out" bad cows until after the second calf is born. In any case it is obviously a matter of some practical importance that the limits of its accuracy should, so far as is possible, be ascertained.

The present work is based on 336 cows which have had five or more calves (about 2240 lactation records in all). Every cow is included in every calculation unless suffering from abortion or serious illness.

The "revised maximum" has been used throughout as the measure expressing a cow's yield for any given lactation. It may be defined as the maximum day-yield of the lactation which is three times reached (or exceeded)<sup>1</sup>. The advantages of using this figure, and its relationship to more ordinary methods of measurement as by lactation totals, have been discussed in a previous paper<sup>2</sup>.

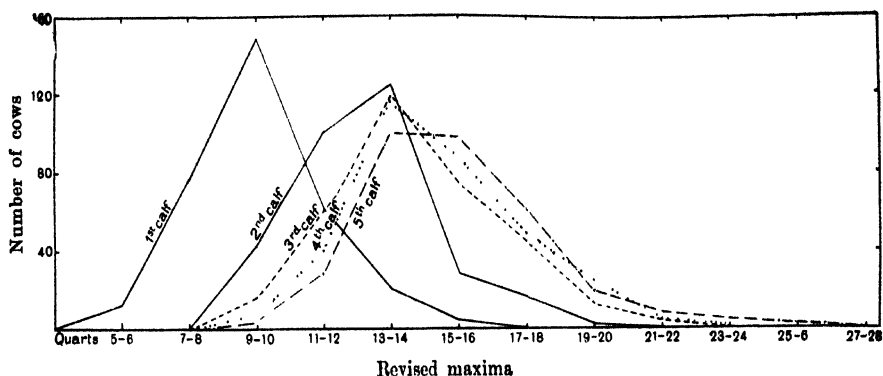
The cows represented are principally British Holsteins and non-pedigree dairy Shorthorns.

The revised maxima given with successive calves were first considered separately, i.e. all the first calf R. M. together, all the second calf R. M.,

<sup>1</sup> In other words, the highest figure common to the three highest day-yields of a lactation.

<sup>2</sup> Gavin, "The Interpretation of Milk Records," *Journ. Roy. Agric. Soc.* 1912, p. 153; v. also "Studies in Milk Records: Influence of Foetal Growth on Yield," *Journ. Agric. Science*, 1913, Vol. v. Part 3.

and so on. The frequency curves obtained for 1st to 5th calf are given in the following diagram.



Frequency curves. Revised maxima. 320 cows.

With first calf ———  
 „ second „ .....  
 „ third „ .....  
 „ fourth „ .....  
 „ fifth „ — . . . . .

Constants of variation of R.M. given with successive calves.

Lactation	No. of records	Mean	Median	Mode	Modal coefficient	Standard deviation	Coefficient of variation
		quarts	quarts	quarts	per cent.	quarts	
First	320	9.3 ± 0.074	9.50 ± 0.092	9.9	47	1.97 ± 0.052	21.2 ± 0.56
Second	313	12.8 ± 0.081	12.77 ± 0.101	12.71	39	2.12 ± 0.057	16.6 ± 0.45
Third	326	14.2 ± 0.091	13.97 ± 0.117	13.51	36	2.53 ± 0.067	17.8 ± 0.47
Fourth	328	14.9 ± 0.091	14.54 ± 0.114	13.82	35	2.46 ± 0.065	16.6 ± 0.44
Fifth	323	15.4 ± 0.104	15.08 ± 0.130	14.44	31	2.73 ± 0.072	17.8 ± 0.47

A class unit of two quarts was taken in all cases.

The mode was calculated from the formula

$$\text{Mode} = \text{mean} - 3(\text{mean} - \text{median}),$$

which gives a reasonably correct value in most cases<sup>1</sup>. The modal coefficient is the percentage of variates falling on the empirical mode, and the coefficient of variation<sup>2</sup> =  $\frac{100\sigma}{\text{mean}}$ .

<sup>1</sup> Pearson, *Biometrika*, Vol. i. 1902, p. 260.

<sup>2</sup> Pearson, 'Regression Heredity and Panmixia,' *Phil. Trans. Roy. Soc.* CLXXXVII. 1896, p. 276.

The usual formulae, which of course can only be said to be strictly correct in cases of normal distribution, were used for calculation of probable errors.

The small variation in the number of cows which could be included in the different lactation calculations (due to abortion or to serious illness at the time when it seemed probable they would have given their maximum yield) has been eliminated in the diagram on p. 378, where all the curves have been reduced to the ratio of 320 cows.

From the table of constants it is seen that the mean continues to increase right up to the fifth calf. Beyond the fifth calf the following figures were obtained :

Lactation	Mean R. M.	No. of cows
Sixth	15.85	221
Seventh	15.51	148
Eighth	15.48	83

but these cannot be strictly comparable with the others, since as soon as one ceases to deal with the same number of cows throughout, the influence of selection must come in. It is probable that only the best or the healthiest of the 336 cows would tend to remain in the herd after their fifth calf.

The coefficient of variation is 21.2 with first calf, after which it falls to the neighbourhood of 17. Determinations made from a smaller number of cows revealed a tendency to rise again after the fifth calf.

The increased variability of first lactation yield seems to the writer more likely to be due to the greatly varying condition in which heifers are brought into the milking shed than to have any physiological basis. Differences in feeding and general treatment and, most of all, in age, must influence very greatly the first lactation yield, and this influence must tend to decrease as the milking career advances.

With uniform treatment and uniform age it seems probable therefore that this extra variability could at any rate be reduced, but for the general conditions of farm practice it must be recognised as a factor affecting the accuracy of estimates based on first lactation yields.

In considering the correlation between the first and subsequent lactation yields, it becomes necessary to decide on a figure to represent a cow's mature capability. The writer has previously proposed taking the average 4th, 5th, and 6th calf R. M., but he now suggests substituting

for this the *maximum* R. M., that is to say, the largest daily yield ever given in the life of a cow, provided it is reached three times in the same lactation (this stipulation being to avoid abnormally high entries due to late milking, clerical errors, etc.).

The whole idea of taking maximum instead of average yields will perhaps be viewed with disfavour by some practical breeders as being less in accordance with actual results obtained. The importance, however, of aiming primarily at physiological capability must be borne in mind, and it is suggested that, in the same way as the maximum day-yield of any one lactation is the least variable function of the yield of that lactation<sup>1</sup> and probably the most reliable guide to the cow's capacity at the time, so the highest of these maxima to which she attains during her life will be the best and most comparable indication of her physiological capabilities in reasonably favourable circumstances.

The correlation coefficients between this maximum R. M. and the R. M. given with first five lactations are as follows :

Lactation	$r$ with max. R. M.	Probable error
First	+·394	$\pm 0\cdot031$
Second	+·452	$\pm 0\cdot030$
Third	+·506	$\pm 0\cdot028$
Fourth	+·605	$\pm 0\cdot024$
Fifth	+·762	$\pm 0\cdot016$

The average<sup>2</sup> constants of variation for the maximum R. M. are :

Mean	Median	Mode	Modal coefficient	Standard deviation	Coefficient of variation
17·14 qts.	16·70 qts.	15·82 qts.	32 %	2·88	16·8

It will be noticed that the second lactation figure is only ·058 higher than that given by first lactation, but this increase does not fully represent the accuracy that can be gained by keeping a cow until after

<sup>1</sup> *v.* Interpretation of Milk Records.

<sup>2</sup> Owing to the fact that all cows were not available for every correlation table, these constants shew slight variations. The actual values obtained were as follows :

Mean. ....	17·0	17·1	17·2	17·2	17·2
$\sigma$ .....	2·85	2·75	2·98	2·95	2·94
$v$ .....	16·8	16·1	17·3	17·2	17·1

*Correlation table. First calf R.M. and max. R.M.*

1st R.M. Quarts	Max. R.M. Quarts								Totals
	12-13	14-15	16-17	18-19	20-21	22-23	24-25	26-27	28-29
5-6	—	2	3	—	—	—	—	—	11
7-8	5	30	26	13	1	—	—	—	75
9-10	6	42	52	34	8	3	1	1	149
11-12	1	5	16	14	16	2	—	3	59
13-14	—	5	8	3	2	1	2	—	21
15-16	—	—	—	2	2	—	—	—	4
17-18	—	—	—	—	—	1	—	—	1
Totals	12	90	105	66	29	7	3	4	320

*Correlation table. Second calf R.M. and max. R.M.*

2nd R.M. Quarts	Max. R.M. Quarts								Totals
	12-13	14-15	16-17	18-19	20-21	22-23	24-25	26-27	28-29
9-10	4	16	12	5	5	—	—	—	42
11-12	4	38	40	15	3	1	—	—	101
13-14	2	29	39	30	17	2	2	—	123
15-16	—	1	12	10	4	—	—	—	28
17-18	—	—	1	4	6	2	—	2	16
19-20	—	—	—	—	—	1	1	—	2
21-22	—	—	—	—	—	1	—	—	1
Totals	10	84	104	64	35	7	3	3	313

*Correlation table. Third calf R.M. and max. R.M.*

3rd R.M. Quarts	Max. R.M. Quarts								Totals
	12-13	14-15	16-17	18-19	20-21	22-23	24-25	26-27	28-29
9-10	4	6	5	1	—	—	—	—	16
11-12	5	30	11	8	4	—	1	1	60
13-14	5	39	48	22	7	—	1	—	118
15-16	—	12	32	17	7	6	—	—	74
17-18	—	1	11	16	9	1	1	1	43
19-20	—	—	—	2	6	1	—	1	11
21-22	—	—	—	—	2	1	—	—	3
23-24	—	—	—	—	—	—	—	—	—
25-26	—	—	—	—	—	—	—	—	—
27-28	—	—	—	—	—	—	—	1	1
Totals	14	88	102	66	35	9	3	4	326

*Correlation table. Fourth calf R.M. and max. R.M.*

4th R. M.	Max. R. M. Quarts								Totals	
	Quarts	12-13	14-15	16-17	18-19	20-21	22-23	24-25		26-27
9-10	2	4	—	1	—	—	—	—	—	7
11-12	7	20	10	3	—	—	—	—	—	40
13-14	3	53	37	18	3	—	—	1	—	115
15-16	—	12	43	15	12	3	2	—	1	88
17-18	—	—	14	22	6	3	1	—	3	49
19-20	—	—	—	7	13	1	—	2	1	24
21-22	—	—	—	—	3	1	—	1	—	5
Totals	12	89	104	66	37	8	3	4	5	328

*Correlation table. Fifth calf R.M. and max. R.M.*

	Max. R.M. Quarts								Totals	
5th R.M. Quarts	12-13	14-15	16-17	18-19	20-21	22-23	24-25	26-27	28-29	
9-10	2	3	—	—	—	—	—	—	—	5
11-12	4	16	6	2	—	—	—	—	—	28
13-14	5	49	35	9	1	1	—	—	—	100
15-16	—	20	48	20	10	—	—	—	—	98
17-18	—	—	11	31	15	2	—	—	—	59
19-20	—	—	—	5	8	—	1	1	3	18
21-22	—	—	—	—	4	2	1	1	1	9
23-24	—	—	—	—	—	2	1	—	—	3
25-26	—	—	—	—	—	—	—	2	—	2
27-28	—	—	—	—	—	—	—	—	1	1
Totals	11	88	100	67	38	7	3	4	5	323

her second calf. By taking the average<sup>1</sup> between 1st and 2nd R.M., the correlation coefficient with max. R.M. is raised to

$$+ \cdot 526 \pm 0.028.$$

<sup>1</sup> Constants of variation given by this average are: Mean 11.18 quarts  $\pm 0.071$ ,  $\sigma$  1.82 quarts  $\pm 0.050$ ,  $v$  16.3  $\pm 0.448$ .

It should perhaps be emphasised that, in calculating this correlation, all available cows were used, as in the other cases. The cows used for determining the correlation of max. R.M. with R.M. of first lactation, of second lactation, and mean of first and second lactations are therefore not all the same (cf. variation in the constants for max. R.M. in the

It would appear that in some cases an abnormally low or high yield (abnormal, that is, for the cow in question—out of proportion, by reason of exterior circumstances, to its inherent capacity) is compensated for by an increase or reduction in the following lactation. Large fluctuations from year to year, even after a cow has reached maturity, are also very common indeed. Many of these would doubtless be explicable at the time by those having full knowledge of the attendant circumstances, but all must reduce the value of the correlation coefficients.

That these tend to be low, even between successive lactations, is shewn by the following results:

Correlation between	<i>r</i>	Probable error
First and second lactations	+ .437	+ 0.031
Second and third    ,,	.388	0.033
Third and fourth    ,,	.576	0.025
Fourth and fifth     ,,	.527	0.028

Thus compared with other relationships, that existing between 1st R. M. and max. R. M. assumes greater importance than the coefficient of .394 would by itself indicate.

With regard to the estimation of mature from first calf yield, a simple factor will not suffice, since cows starting badly tend to increase to a greater proportion of their first calf yield than those which begin with a higher figure. Recourse must therefore be had to regression coefficients<sup>1</sup>, which are given in the following table, together with the probable error of estimate<sup>2</sup>.

footnote on p. 380). As a consequence if we calculate the s.d. of mean R. M. of first and second lactations from the formula  $\frac{1}{2}(\sigma_1^2 + \sigma_2^2 + 2r_{12}\sigma_1\sigma_2)^{\frac{1}{2}}$ , we get 1.73, not 1.82: and if we calculate the correlation from the formula

$$\frac{r_{11}\sigma_1 + r_{21}\sigma_2}{\sqrt{\sigma_1^2 + \sigma_2^2 + 2r_{12}\sigma_1\sigma_2}},$$

we get 0.500, not 0.526. By reason of the coefficients not being entirely comparable with each other, the application of the theory of partial correlation to determine a regression equation giving the deviation in max. R. M. in terms of the deviations of first and second R. M. separately is a little doubtful. Mr Yule finds the equation

$$x_m = 0.345 x_1 + 0.457 x_2,$$

where  $x_m$  is the deviation in max. R. M. The probable error of estimate in using this equation is only very slightly lower than the p.e. of estimate in using the mean of first and second lactations.

<sup>1</sup> Regression coefficient of  $x$  relative to  $y = r \frac{\sigma_x}{\sigma_y}$ . Probable error of regression coefficient =  $\frac{.6745\sigma_x}{\sigma_y} \sqrt{\frac{1-r^2}{n}}$ .

<sup>2</sup> Probable error of estimate =  $.6745 \times \sigma \sqrt{1-r^2}$ .



*Regression of max. R.M. relative to R.M. 1—5.*

Lactation	Regression coefficient	Probable error of estimate
		quarts
First .....	$\cdot 57 \pm 0\cdot 050$	1\cdot 77
Second .....	$\cdot 58 \pm 0\cdot 046$	1\cdot 66
Third .....	$\cdot 60 \pm 0\cdot 037$	1\cdot 73
Fourth .....	$\cdot 73 \pm 0\cdot 035$	1\cdot 58
Fifth .....	$\cdot 82 \pm 0\cdot 026$	1\cdot 28
Average first and second ...	$\cdot 79 \pm 0\cdot 053$	1\cdot 58

One example may be given of the use of the regression coefficient. A cow gives R.M. 7·3 quarts with first calf. What will be her max. R.M.? The mean of first calf R.M. is 9·3, i.e. she differs from that mean by -2. She will therefore differ from the mean of max. R.M., which is 17·14 quarts, by  $-2 \times$  regression coefficient, that is, her average, or expected, max. R.M. will be

$$17\cdot 14 - (2 \times \cdot 57) = 17\cdot 14 - 1\cdot 14 = 16 \text{ quarts.}$$

Further, the chances are *even* that this estimate is correct within the limits of  $\pm 1\cdot 8$  quarts.

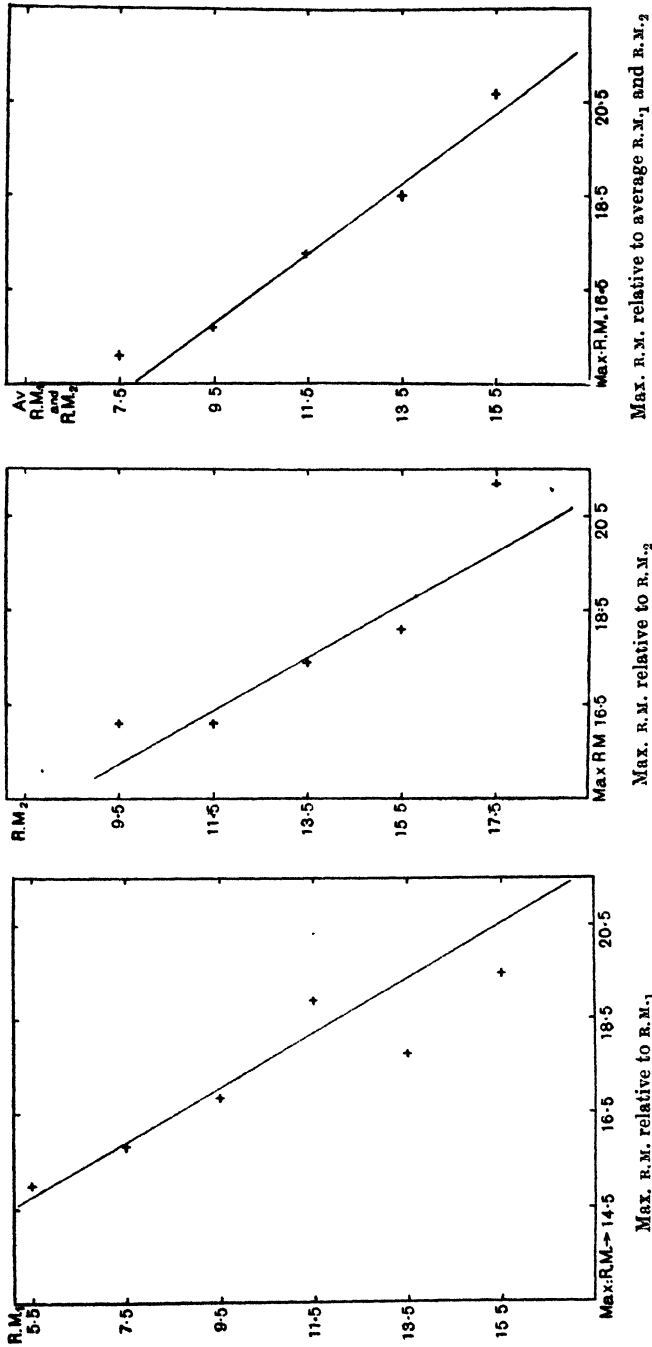
The accuracy of these calculations depends on the distribution of each array being normal, and on the means of arrays falling on the line representing characteristic regression. Unfortunately neither of these conditions are perfectly fulfilled, and the results cannot be regarded as more than the greatest approximation to accuracy so far obtained.

The following diagrams shew the line of characteristic regression, together with means of arrays, for max. R.M. relative to (1) first calf R.M., (2) second calf R.M., and (3) average between first and second calf R.M. It will be seen that with (3) the regression is very nearly linear.

The results of the application of the regression coefficients are given in tabular form. A column has been added shewing the calculated best totals for a normal lactation. These have been derived from constants previously obtained in calculating the correlation between totals and R.M. of 1233 cows<sup>1</sup>. These were as follows:

1233 normal lactations		
Mean. Totals .....	656·4 gals. $\pm 3\cdot 24$	
Correlation coefficient .....	$\pm 0\cdot 87$ gals. $\pm 0\cdot 01$	
Regression coefficient .....	$\pm 844$ $\pm 0\cdot 005$	
Totals relative to R.M. ....	41·54 $\pm 0\cdot 518$	
Probable error of estimate .....	$\pm 60$ gals.	

*Lines of characteristic regression, with means of arrays.*



Thus, in the following table, column 4 represents the *best* yield likely to be obtained from a cow giving the corresponding first calf R.M. (column 1) in a normal lactation of about 9 months, at an average season of calving. Cows calving from September to April would be inclined to yield about 50 gallons more, and those calving from May to August up to 50 gallons less<sup>1</sup>.

*Table for estimating max. R.M. from (a) 1st R.M. and (b) average 1st and 2nd R.M.*

1st calf R.M.	Calculated max. R.M.	Limits of probable error	Lactation total <sup>2</sup> corresponding to max. R.M.
quarts	quarts	quarts	gallons
5	14.6	12.9—16.3	685
6	15.1	13.4—16.8	709
7	15.7	14.0—17.4	733
8	16.3	14.6—18.0	757
9	16.8	15.1—18.5	781
10	17.4	15.7—19.1	804
11	18.0	16.3—19.7	827
12	18.5	16.8—20.2	851
13	19.1	17.4—20.8	874
14	19.7	18.0—21.4	898
15	20.3	18.5—21.9	921
16	20.8	19.1—22.5	944
Average R.M. 1st and 2nd calves			
7	13.7	12.1—15.3	651
8	14.5	12.9—16.1	684
9	15.3	13.7—16.9	717
10	16.1	14.5—17.7	750
11	16.9	15.3—18.5	783
12	17.7	16.1—19.3	816
13	18.5	16.9—20.1	849
14	19.3	17.7—20.9	882
15	20.1	18.5—21.7	915
16	20.9	19.3—22.5	948

The next table has been compiled from an analysis of the correlation between max. R.M. and R.M.<sub>1</sub>. The former have been converted into the corresponding lactation totals, and the percentage of cows calculated which gave max. R.M. corresponding to less than 700, 700—800 and more than 800 gallons respectively. It must be emphasised that these

<sup>1</sup> v. Interpretation of Milk Records.

<sup>2</sup> The probable error of estimated lactation total is about  $\pm 61$  gallons, and is expressed by the formula  $\sqrt{(\text{p.e.})_1^2 + (\text{r.c.})^2 (\text{p.e.})_2^2}$ , where  $(\text{p.e.})_1$  is P.E. of estimate of max. R.M.,  $(\text{p.e.})_2$  the P.E. of estimate of lactation total from any R.M., and r.c. the regression of totals relative to R.M. The author is indebted to Mr G. Udny Yule for information on this point.

are the *largest* yields the cows are ever likely to give in a normal lactation; their average yields would of course be considerably less. Such estimates must necessarily be approximate, but they may serve as a guide to the general proportions to be expected.

Cows giving maximum total yield of			
1st calf R.M.	Less than 700 gals.	700—800 gals.	More than 800 gals.
	%	%	%
5—7 quarts .....	33	50	17
8—9 „ .....	22	54	24
10—11 „ .....	14	45	41
12—17 „ .....	8	29	63
Less than 10 quarts	25	53	22
10 quarts.....	16	48	36
More than 10 quarts	8	33	59

One other method of examination has been employed. The cows were divided into three classes according to their first calf R.M. The mean R.M. with subsequent calves was then calculated for each class. The results are given in the following table and diagram (page 388).

1st calf R.M.		Mean R.M.					Average 1st—5th	
		No. of cows	1st	2nd	3rd	4th	5th	R.M.
			qts.	qts.	qts.	qts.	qts.	Corresponding normal lactation total
Class C.	5—9 qts.	153	8.1	11.3	13.7	14.4	15.4	12.6
„ B.	10—11 „	112	10.4	13.3	14.7	15.3	15.9	13.9
„ A.	12—17 „	50	13.0	14.9	16.1	16.4	16.9	15.5
								gallons
								604
								658
								724

These figures lead to some very significant conclusions. The average difference between classes C and B is 54 gallons, or a total of 270 gallons in the five years. At 8d. a gallon this comes to £9. Similarly calculated, the difference between classes A and B is £11. Thus, according to the present data an *average* cow of class A should give a return of £20 in five years over and above that given by an *average* cow of class C.

These three divisions of first calf R.M., namely (c) less than 10 quarts, (b) 10 and 11 quarts, and (a) 12 quarts and upwards, should be useful ones for the practical breeder to bear in mind. It certainly appears that cows of class C should be discarded, as far as possible, after their first calf, bearing in mind however the increased accuracy of a judgement

based on the mean of first and second calf R.M. Thus if any extenuating circumstances are present, a reprieve should be granted until after the second calf. The probability is that about one in five will turn out good yielders.

Class B offers the great opportunity to the skilful judge of dairy-cows. It is "odds on" that they will pay for keeping, but "odds against" that they will turn out high-yielders. Speaking generally, these cows should be kept for their second calf.

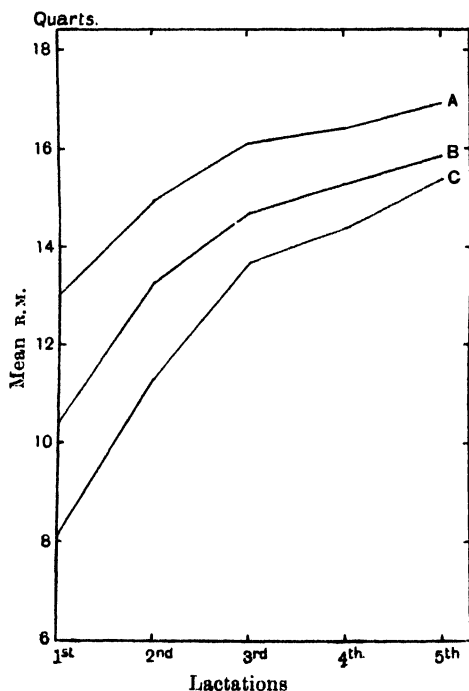


Diagram shewing subsequent average yield of cows classed as follows :

Class A.	Cows giving 1st R.M. of 17—12 quarts.
" B.	" " 11—10 "
" C.	" " 9—5 "

Cows of class A no dairy farmer will be likely to part with, and in spite of individual exceptions it is clear from the preceding diagram that cows starting well tend on the whole to maintain a good proportion, though not all, of their initial lead over the bad starters. At the same time it unfortunately appears to be less certain that class A will do well than that class C will do badly, as shewn by the following coefficients of variation :

Variability of max. R.M. given by cows belonging to

Class C	Class B	Class A
$13.5 \pm 0.51$	$17.2 \pm 0.78$	$16.9 \pm 1.14$

Classed according to 1st calf R.M.

There is another field for the practical application of such results. In the study of the inheritance of milk yield it becomes necessary to define a cow's mature capability by a single unqualified figure, and it has already been suggested that the max. R.M. should be chosen for this purpose.

In breeding, however, this implies many years delay, and in the examination of records the number available becomes very greatly reduced if all cows that did not reach maturity have to be excluded. It is advisable therefore that a uniform system should be formed to deal with the immature cows by calculating the max. R.M. from the data available in each case.

It is probable that calculation will give in many cases a less accurate result than a prognostication by a practical breeder, but in such work it seems essential that personal bias should be excluded.

### SUMMARY.

(Lactations are measured throughout by R.M. in quarts.)

1. The first lactation shews greater variability than the second, third, fourth or fifth.
2. The estimation of one lactation from another cannot be made with great accuracy, since the correlation coefficient between even successive lactations does not rise above +.6.
3. It is necessary, in classing a cow, to decide on one lactation that shall represent her mature capability. It is suggested that the maximum lactation is the most suitable one to chose.
4. The correlation coefficient with the maximum lactation increases from .394 for first to .762 for fifth lactation.
5. The mean of the first and second lactations however gives a correlation coefficient of .526 with maximum lactation, which is higher than any of the first three taken separately.

6. The probable error of estimating maximum from first, second or third lactations is about 1·7 quarts. The inaccuracy is likely to be greater than this, since regression of the former has not yet been shewn to be strictly linear.

7. The mean of first and second lactations gives a probable error of estimate of 1·6 quarts and the regression of maximum lactation relative to this figure is very nearly linear.

8. Tables are given for estimating maximum lactation from both first lactation, and mean of first and second.

9. The general conclusion arrived at is that with cows giving a fairly high or fairly low first lactation R.M., this figure should be used to determine whether they shall be kept or not; but with cows giving a medium first lactation R.M. of 10—11 quarts, it is worth waiting to obtain the increased accuracy of an estimate based on the mean of first and second lactation R.M.

These investigations are being undertaken on behalf of Lord Rayleigh and the Hon E. G. Strutt with data accumulated by them during the last twenty years. For any deficiencies in method or treatment of the material, however, the author is alone responsible.

## THE DISTRIBUTION OF ATMOSPHERIC IMPURITIES IN THE NEIGHBOURHOOD OF AN INDUSTRIAL CITY.

By CHARLES CROWTHER, M.A., PH.D., AND  
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IN a previous paper<sup>1</sup> a summary was given of the results obtained in an examination of the degree of pollution of the atmosphere in various parts of the city of Leeds, as diagnosed by the analysis of samples of rain collected at various centres. These results showed that, even in the cleaner suburbs of the city, the purity of the air compares very unfavourably with the standard of country districts remote from industrial areas.

An examination in similar fashion of the atmosphere in the country surrounding the city to a distance, in some directions, of seven miles from its centre, has now been completed, and the results are summarised and discussed in the following pages.

Throughout the twelve months from July 1, 1911, to July 1, 1912, samples of rain were collected at fourteen stations situated respectively with reference to the centre of Leeds as indicated in the following schedule (Table I) and diagram (Fig. 1).

Briefly summarised, the stations were so arranged as to give a complete ring of stations on a circle of three miles radius from the centre of the city, whilst to the north, north-east and east additional stations were selected at distances of five miles and seven miles respectively from the centre. Leeds is bordered in all directions, except the three above-mentioned, by a thickly populated industrial area, coal-mining and iron-working prevailing to the south-east and south, and woollen manufacturing to the south-west and west, whilst

<sup>1</sup> Crowther and Ruston, *This Journal*, iv. pp. 25—55.



large engineering works are situated throughout both areas. It was felt therefore that little was to be gained by collecting samples more than three miles out in these directions, since the existence of heavy and varied local pollution would almost hopelessly complicate the interpretation of the results. Thus, a station seven miles due west of Leeds would be well within the boundaries of the almost equally large industrial city of Bradford; at the same distance to the south-west is the heart of one of the chief manufacturing areas of Yorkshire—the Heavy Woollen District—whilst to the south at a distance of about eight miles lies the city of Wakefield. It requires no special

TABLE I. *Location of Collecting Stations.*

Direction from Centre	Distance from Centre	Name of Locality
	miles	
N.	7	Harewood
N.	5	Alwoodley
N.	3	Meanwood
N.E.	7	Thorner*
N.E.	5	Shadwell
N.E.	3	Roundhay
E.	7	Garforth
E.	5	Manston
E.	3	Seacroft
SE.	3	Rothwell
S.	3	Middleton
S.W.	3	Gildersome
W.	3	Bramley
N.W.	3	Kirkstall

\* It was necessary to abandon the collecting station first selected in this district, as all the samples of rain obtained showed unmistakable evidence of excessive pollution from the village of Thorner, which lay close by to windward of the collecting station (see Fig. 1).

investigation to demonstrate that throughout the whole of this area and much farther afield in these directions the atmosphere is universally polluted by smoke to a very appreciable extent.

On the other side of Leeds, however, there are no such complications, as the country to the north, north-east and well down towards the east is purely agricultural in character.

Any general atmospheric pollution of the character of smoke that prevails over this area may thus be ascribed with a high degree of certainty to the chimneys of Leeds or of remoter industrial centres, and should show a marked gradation at points successively more remote from the town. In selecting stations at which the funnels could be

kept conveniently under observation difficulty was experienced in some cases in evading pollution from the smoke of the neighbouring village. Reference has already been made to the case of Station N.E. 7 (a), and we have reason to believe that the results obtained at Stations N.E. 5 and E. 5 were also unduly high for similar reasons (Fig. 1).

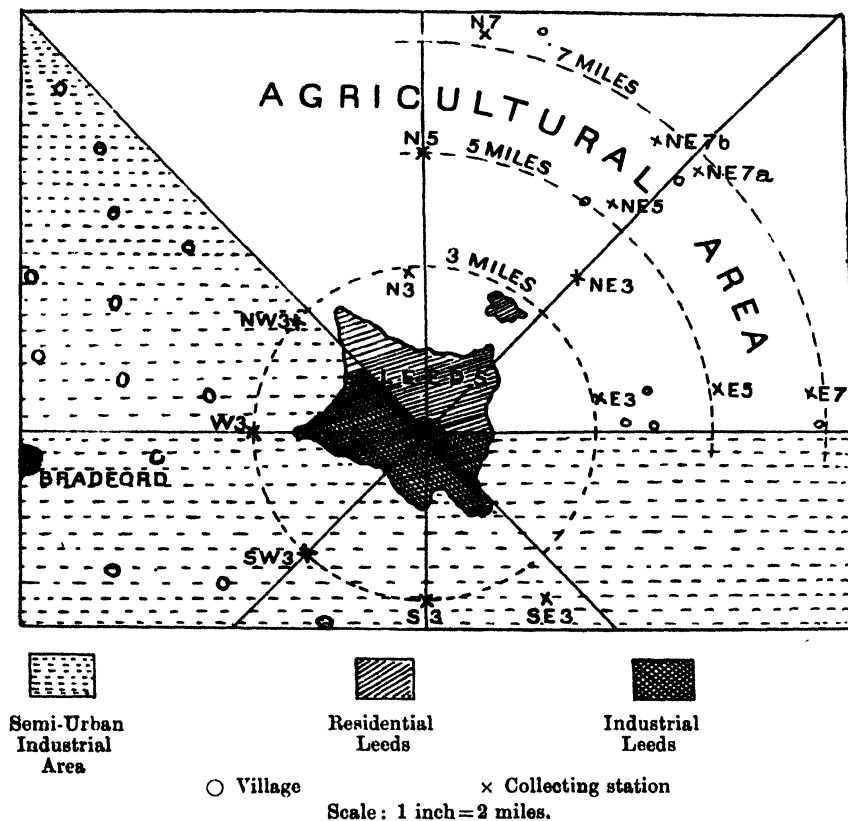


Fig. 1. Showing positions of collecting stations with regard to Leeds and nature of surrounding areas.

At each station the samples were collected by means of a copper funnel, 15 inches in diameter, with vertical upper walls 3 inches deep, the funnel being supported on iron legs so that its rim was 18 or 20 inches above the surface of the ground. Under the funnel was placed a large bottle standing in a wooden box. The capacity of the bottle was  $4\frac{1}{2}$  litres, so that it would accommodate a rainfall of roughly  $1\frac{1}{2}$  inches. A scale was marked on the side of the bottle in order that

it might serve as a rain-gauge. When the bottle was nearly full, the rainfall contained in it was read off, the whole thoroughly shaken and a quantity of about two litres rapidly transferred to a clean Winchester bottle. The remainder of the rain collected was then thrown away and the large bottle replaced under the funnel.

The arrangements made for obtaining the samples in this way would have been adequate in a normal year, but were strained to the utmost by the abnormal weather conditions of 1911-12.

With the assistance available fully two days were required in order to do the round of the funnels whenever it was necessary to remove the samples, and on a few occasions rain fell so heavily and persistently that some of the collecting bottles overflowed before they could receive attention.

Further irregularities in the records occasionally arose owing to the impossibility of constant supervision of the funnels, a few samples being rejected owing to obvious undesirable pollution not of atmospheric origin, and a few other samples lost through fracture of the collecting bottles by frost, or otherwise.

In making up the totals for the year it has been necessary therefore to compute the composition of these missing samples, and this has been done by close comparison of the records of the neighbouring stations. The data assessed in this way amount to about 7 per cent. of the total number.

For these and other reasons indicated later we do not profess to have secured records of high absolute accuracy, but we regard them as amply reliable for the comparative purposes with which alone our work is concerned.

In order to ensure strict comparability of the analytical results the whole of the analyses were undertaken by one of us (D. W. S.). The range of variation in the individual samples and the probable errors of the averages are given in the Appendix, p. 408.

The following table (Table II) gives a summary of the results for the twelve months, the totals being expressed for convenience as pounds per acre. In all, 291 samples were dealt with. Twelve further samples should have been obtained, but were lost through breakage of bottles or other unavoidable cause.

No close examination of the table is necessary to discover that very marked and significant differences are shown between the various areas. It will be observed that at all the stations on the three-mile ring the pollution was high, notably in suspended matters and sulphur—the

two outstanding features of smoke pollution. Further, the more pronouncedly industrial districts (from S.E. 3 downwards) show much greater pollution than the districts equidistant from the centre of the city lying to the north and north-east.

TABLE II. *Total impurities of various kinds brought down by rain during the twelve months, July 1911—June 1912.*

Collecting station	Rainfall	(Pounds per acre)					
		Total suspended matters	Suspended mineral matter		Sulphur expressed as SO <sub>3</sub>	Chlorine	Nitrogen
			Total	as per cent. of total susp. matter			
	inches	lbs.	lbs.	per cent.	lbs.	lbs.	lbs.
N. 7 *	33·2	72	38	53	128	44	6·7
N. 5	33·1	104	57	55	192	59	9·1
N. 3	30·1	175	107	61	218	53	5·9
{ N.E. 7 (a)†	31 0	131	78	60	241	53	8·5
{ N.E. 7 (b)†		121	73	60	129	40	5·8
{ N.E. 7 (c)†		126	75	60	162	46	6·6
N.E. 5	29·4	150	92	61	168	43	9·1
N.E. 3	30·5	120	62	52	186	45	8·5
E. 7	29·1	122	63	51	168	43	6·6
E. 5	28·7	212	106	50	171	50	8·0
E. 3	27·8	200	109	54	207	54	7·9
S.E. 3	25·8	353	250	71	357	63	8·9
S. 3	28·2	286	154	54	269	47	9·3
S.W. 3	32·7	239	149	62	268	56	8·0
W. 3	30·4	292	178	61	284	56	8·7
N.W. 3	28·3	194	110	57	380	70	7·4

\* It will be convenient to describe the stations in this fashion, "N. 7" being the station situated 7 miles to the north; "S.E. 3" the station situated 3 miles to the south-east, and similarly throughout.

† See footnote, p. 392. Station (a) is the station first selected in this area and abandoned on Nov. 21st, 1911, in favour of Station (b). The data given for (a) and (b) are computed on the assumption that the collection of samples had continued at (a) or (b) respectively for the whole of the 12 months. The data given under 7 (c) are the totals for the year as actually obtained.

Further, there is an obvious sharp falling-off in general pollution on passing away from the city northwards or north-eastwards, but a much more gradual fall in passing eastwards. The influence of the prevalent westerly winds in disseminating the smoke is thus brought out in very interesting fashion, although there is a certain degree of

complication in that this easterly line of stations is situated just on the northern fringe of the coal-mining area.

One further item of general interest may be singled out before passing to a more detailed consideration of the results—the relatively high degree of pollution even in the cleanest direction at a distance of seven miles from the city<sup>1</sup>.

#### RAINFALL.

There was an appreciable difference in the rainfall at the different stations, the range in the yearly totals being from 26 inches at Station S.E. 3 to 33 inches at the more remote northerly stations—a variation of more than 20 per cent. The higher rainfalls were recorded on the more hilly side of the city, commencing with south-west and working round by west to north.

#### SUSPENDED MATTER.

It has proved almost impossible to obtain an accurate measure of the total suspended matter of atmospheric origin falling at each station. In the first place, it was soon found to be necessary to protect the funnels from birds by fixing a screen of black cotton thread over them. Further trouble was experienced at most centres by flies and other insects, fragments of leaves, etc., finding their way into the samples, and the difficulty was only partially overcome by placing discs of copper gauze of close mesh in the funnels. It is further possible that at some centres (*e.g.* S.E. 3) the funnels may have received an abnormal amount of dust owing to the proximity of roads or cultivated land.

This may to some extent account for the high proportion of mineral matter in the total suspended matters at these stations, but at the same time the fact must not be overlooked that most of the stations where the proportion of ash in the total suspended matters is above the average (56 per cent.) are close to the industrial area, where a high proportion of grit in the smoke might be expected.

<sup>1</sup> It is of interest to note that the proportion of impurities contained in the samples collected during the period of the great coal miners' strike (March, April, 1912) was decidedly below the normals for the periods immediately preceding and following it—especially in the industrial area. This can scarcely be regarded as a coincidence, and it is probable that in a year with normal output of smoke the yearly totals might be appreciably higher than we have found.

Despite irregularities in individual samples due to the causes above outlined, the results as they stand are sufficiently precise to bring out the marked difference between the "industrial rural" area to the south and west and the agricultural area to the north and north-east. Moreover they indicate the steady falling-off to the north with increased distance from the city, whilst to the north-east and east the falling-off is not so pronounced. The results for Stations N.E. 5 and E. 5 are abnormal in this respect, being higher than those for the stations nearer to the city, N.E. 3 and E. 3 respectively. The high proportion of ash affords an explanation in the former case, whilst at the latter station, owing to the proximity of trees and inadequate local supervision, great trouble was experienced with birds and leaves.

The grading of the stations comes out rather more sharply if made on the basis of the ash-free suspended matters, as may be seen from the following chart:—

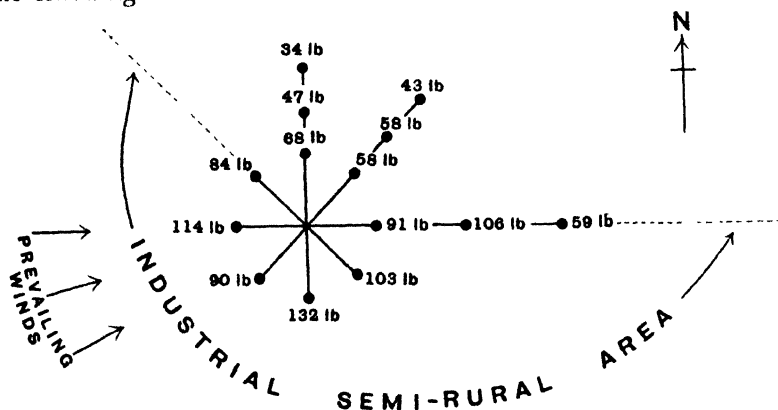


Fig. 2. Chart showing the amounts (lbs. per acre per annum) of ash-free solids brought down at various stations.

It is clear from the chart that the results in general agree closely with what might be expected from a comparison of the relative positions of the stations with regard to the smoke-distributing area.

"Tar." In the majority of the samples taken during the first six months estimations were made of the ether-soluble matter ("tar") contained in the suspended matters, with results which may be summarised as follows:—

*Percentage of tar in total suspended matters.*

	Clean districts	Dirty districts
Maximum	15.8 (Station N. 7)	10.9 (Station W. 3)
Minimum	6.8 ( " N. 3)	4.8 ( " S.E. 3)
Average	9.5 ± .7	7.6 ± .2

These results are quite in harmony with expectations since in industrial districts a considerable proportion of the coal consumed is subjected to a much more thorough combustion than in rural districts, where the smoke arises mainly from domestic fires whose imperfect combustion gives rise to soots rich in tar<sup>1</sup>.

#### SULPHUR.

In every sample, after removal of the suspended matters, the total sulphur was determined by precipitation with barium chloride after boiling with bromine water—the determinations being made throughout in duplicate.

The results are summarised in Table II, and are surprisingly high all round, ranging from 128 lb. at Station N. 7 to 380 lb. at Station N.W. 3. These figures are even higher than those obtained in 1907–8 at some of the stations within the city<sup>2</sup>, although this difference is doubtless partly accounted for by the decidedly heavier rainfall of the later period.

A study of the results leaves no doubt that the sulphur-content of the rain samples is the most characteristic indication of the degree of pollution of the atmosphere with coal smoke. The lowest amount recorded at stations on the three miles radius was 186 lb., at Station N.E. 3, a quantity two-and-a-half times as great as was found in the earlier investigations for the same locality! The two sets of observations are in agreement, however, in indicating that the sulphur-content of the atmosphere at this distance from the centre of the city is lower in this north-easterly direction than in any other.

On passing further away from the city, however, the sulphur falls most rapidly in the northerly direction (218 lb. to 128 lb.), less in the north-easterly direction (186 lb. to 129 lb.), and still less towards the east (207 lb. to 168 lb.).

The totals for the stations on the industrial side of the city are uniformly high, and such differences as are recorded must be attributed largely to the varying degree of unavoidable local smoke pollution. Thus the higher figure obtained at Station N.W. 3 than at W. 3 finds an obvious explanation in the presence to the west of the former within a distance of about half a mile of large ironworks producing

<sup>1</sup> Cf. Crowther and Ruston, *This Journal*, iv. 38; Cohen and Ruston, *Journal Soc. Chem. Ind.* xxx. (1911), 1360.

<sup>2</sup> Crowther and Ruston, *loc. cit.* 34, 40.

great volumes of smoke, whilst a line of railway also runs in the near neighbourhood.

*Sulphur present in forms other than Sulphate.* In the majority of the samples collected during the first six months, determinations were made not only of the total sulphur, but also of the sulphur present as sulphate, the difference then representing the sulphur present in lower stages of oxidation. The amounts of rain available for these estimations were, however, too small to admit of this difference being determined with great precision. We would merely record therefore that the average of all samples indicated that 6.9 % of the sulphur was present in forms other than sulphate, the averages for the clean and dirty districts being 7.6 ( $\pm 2$ ) and 6.0 ( $\pm 8$ ) per cent. respectively. Excluding doubtful data, the highest proportion (10.4 %) was found at Station N. 7 and the lowest (4.6 %) at S.E. 3.

The higher figure obtained for the relatively clean districts must be attributed to the prevalence there of smoke of domestic origin (cf. also tar, p. 397). It is possible that, contrary to the commonly held opinion, the smoke arising from the combustion of coal under industrial conditions may in some cases contain its sulphur not mainly as sulphur dioxide but almost entirely in the form of sulphate. This was brought out clearly by the investigations of Herbig, which indicated that not more than  $\frac{1}{100}$  of the sulphur of the coal left the chimney as  $\text{SO}_2$ .<sup>1</sup>

#### CHLORINE.

The chlorides present in the rain samples were determined by titration with silver nitrate solution in presence of potassium chromate. The presence of traces of copper (from the funnels) masked the end-point unless a slight excess of the indicator were added and the liquid filtered before titration.

It will be seen that the range of variation between the different stations (from 40 lb. at N.E. 7 to 70 lb. at N.W. 3) was much less with regard to chlorine than in the case of sulphur. There is still evident, however, the differentiation, although not so sharp, between the industrial and the agricultural areas. There are also indications of a falling-off in the amount of chlorine on passing away from the city.

The chlorine, however, does not appear to be as reliable a guide to smoke pollution as the sulphur.

<sup>1</sup> W. Herbig, *Zeits. angew. Chem.* xxii. 1882.



## NITROGEN.

In the earlier samples separate determinations were made of the nitrogen present as ammonia, nitrate, and in "albuminoid" form—the last-named being the nitrogen expelled as ammonia on boiling the water (freed from ammonia) with alkaline permanganate. In the later samples the three determinations were combined in one to obtain simply the total nitrogen present. The results were probably a little lower than would have been obtained by determining the three forms separately.

The yearly totals given in Table II range from 5·8 lb. (Station N.E. 7) to 9·3 lb. (Station S. 3), but there is a lack of the regularity in the gradation of the stations that is evident in the sulphur figures.

The nitrogen results showed two periods of abnormal figures, viz. at the beginning and end of the twelve months. The former we attribute to the pollution of the samples at that period (July, August, 1911) by the droppings of birds (cf. p. 396), but for the latter we cannot trace the cause, unless it be that the prevalence of severe thunderstorms during May and June, 1912, caused unusual enrichment of certain samples with nitrogen.

If the results for these two periods be eliminated the totals for the remainder of the year are as follows:

Station	N. 7	—	3·3 lbs. per acre
	N. 5	—	3·9
	N. 3	—	3·75
	N.E. 7	—	3·7
	N.E. 5	—	4·9
	N.E. 3	—	3·7
	E. 7	—	3·8
	E. 5	—	4·3
	E. 3	—	4·1
	S.E. 3	—	6·0
	S. 3	—	5·9
	S.W. 3	—	5·0
	W. 3	—	5·1
	N.W. 3	—	4·4

The difference between the agricultural and industrial areas is now more obvious, the average of the first nine stations being 3·9 lb., as against the average of 5·24 lb. for the remaining five stations, all situated in the industrial area.

*Nitrogen in Different Forms.*

The organic nitrogen (where estimated) ranged from 6 % (Station N.W. 3) to 21 % (Station S. 3) of the total nitrogen.

Nitrate (+ nitrite) nitrogen ranged from 17 % (Station S. 3) to 30·8 % (Station N.W. 3) of the total nitrogen.

Nitrogen present as ammonia ranged from 61 % (Station N. 5) to 75 % (Station N.E. 5).

The averages for all samples in which separate determinations were made were as follows:

	Percentage of total nitrogen present as		
	Ammonia	Nitrate	Organic matter
All stations	67·3	21·5	11·2
9 "clean" stations	67·7	20·7	11·6
5 "dirty" stations	66·8	22·7	10·5

The ratio of ammoniacal nitrogen to nitrate nitrogen is thus on the average of all stations 75·8 : 24·2—almost identical with the ratio obtained in the earlier Leeds investigations, the corresponding ratios for the clean and dirty districts being 76·6 : 23·4 and 74·7 : 25·3 respectively.

The proportion of organic nitrogen, it will be noted, was on the whole slightly lower in the dirty districts than in the "clean" area, an observation which agrees with the correspondingly lower proportion of sulphur in lower stages of oxidation (p. 399).

### FREE ACID.

The samples of rain collected by Crowther and Ruston in Leeds and at Garforth (Station E. 7 of present series) were in many cases distinctly acid to methyl orange.

The reaction of each sample of the present series has therefore been carefully noted, but in only the following cases was an acid reaction obtained:

Station	Total samples	No. of acid samples
N. 3	23	1
E. 7	20	2
S.E. 3	23	2
S. 3	23	3
S.W. 3	20	1
W. 3	21	2
N.W. 3	23	2
Total	153	13

Of the 140 non-acid samples, some were neutral, but most were distinctly alkaline to methyl orange, congo red and litmus.

It has not been possible to obtain a reliable measure of the free acids contained in the rain, owing to their action upon the copper

of the funnel. An attempt was made to get over the difficulty by applying a coat of lacquer to the copper, but it did not prove successful. The neutralisation of acid by the copper was demonstrated by comparisons made at Garforth between rain collected by means of a glass funnel and that collected through the copper funnel. In the two cases where acid samples were obtained through the copper funnel, the rain collected by the glass funnel was distinctly more acid, whilst on two further occasions the "glass funnel samples" were acid, whilst the corresponding "copper funnel samples" were not.

A further difficulty in the way of obtaining reliable determinations of the acidity of the rain lay in its action upon the glass of the collecting bottle. Although the inside of the bottle was soaked in hydrochloric acid for some time and then thoroughly washed before use, tests with waters of known acidity indicated a decrease of acidity during storage. In one case, where the two halves of such a water were put in separate untreated bottles and left overnight, a very marked difference was found on the following morning. There can be little point therefore in recording the amounts of acid actually found in the samples showing acid reaction, as they must have been much below the original acidity of the rain at the time of falling upon the funnels. It is certain that with more satisfactory arrangements for the collection of the samples the presence of free acid would have been more frequently detected.

#### EFFECTS OF SMOKE UPON VEGETATION<sup>1</sup>.

In the previous paper brief reference was made to the effects of smoke pollution in accelerating the fall of leaves from trees. More extensive observations with regard to this and other effects have now been made throughout the area under investigation, and the general results may be briefly summarised.

No general effect upon the opening of the buds of trees could be detected. The differences between trees of the one species in any one district were quite as great as those found between different districts.

The majority of the trees round about the town were almost in full leaf by the beginning of May, but by May 29th smoke damage began to be evident within two miles of the centre of the city by the

<sup>1</sup> For an exhaustive discussion of the most important work on the subject up to 1903 see Haselhoff and Lindau, "Die Beschädigung der Vegetation durch Rauch" (*Gebr. Bornträger, Leipzig, 1903*).

appearance of the leaves—sycamores and limes in particular showing characteristic brown blotches.

On May 30th observations were made in extensive woods near Station S. 3 (Middleton), flanked on the south-east by large collieries and coke-ovens. Very little leaf-staining was found inside the woods, but along the edge nearest to the works there were abundant signs of damage. Young ash leaves were found shrivelled up and blackened. Elms and sycamores were also badly marked. A few thin-leaved sycamores, growing somewhat overshadowed by others, had their leaves hanging limp and yellow, and the edges had disappeared, leaving a rough discoloured fringe.

Throughout the district from Station W. 3 (Bramley) to Station N.W. 3 (Kirkstall) there was at this early period abundant evidence of damage—beech, oak and sycamore showing brown leaf-blotching: birch had brown curled-in edges and elm black shrivelled edges. The damage was intensified in the latter district, owing to the presence of a large forge (cf. p. 398), and sycamore, elm, oak, laburnum, beech, chestnut and others showed leaf damage.

Similarly, at the other end of the more polluted area, near Station S.E. 3 (Rothwell), tree leaves in general were badly stained and often shrivelled up.

Thus, throughout the polluted area to the south and west of Leeds a considerable proportion of the leaves of trees were badly damaged before they had been expanded one month.

Throughout the cleaner districts to the north and north-east no appreciable amount of leaf-damage could be detected at this period.

During June matters became considerably worse throughout the polluted area, and in the case of large numbers of trees the leaves can have been of but little use. For such trees the annual period of growth is thus very short and their progress correspondingly slow. This was confirmed by the examination of sections of oak trees cut down in the woods above-mentioned. The annual rings were found to be very narrow<sup>1</sup>.

With regard to the general comparison of the clean and smoky districts the outstanding features are the small numbers of trees, and indeed the total absence of coniferous trees throughout the latter districts, whereas in the cleaner districts the trees are much larger and carry more and bigger leaves.

<sup>1</sup> Cf. Haselhoff and Lindau, *loc. cit.* 106.

Great differences are found between individual trees of the same species with respect to their powers of resistance against smoke. Even in the polluted districts it is only trees here and there that are dead or have dead tops, branches or twigs. Ash, poplar, apple, elm, beech, and particularly oak and birch, were found thus permanently damaged. Conifers, however, are entirely absent, despite attempts to introduce them. In the cleaner districts Black Austrian pine, Scots pine and spruce were found between Station N.W. 3 (Kirkstall) and N. 3 (Meanwood); pines at Meanwood (N. 3); cedars and several species of pine at Gledhow (N.E. 3); Austrian and Scots pine at Roundhay (N.E. 3); whilst at Seacroft (E. 3) cedars, Scots and Austrian pine grew, but larch and spruce had failed. In one part of this district some large larch trees were found in a sheltered valley, but were said to have made no growth during the last generation, but rather to be "going back owing to the smoke" and hence are to be cut down shortly.

Passing further away from the town, larch—too sensitive nearer the city—was found five miles out to the north-east (Shadwell), whilst at Garforth (E. 7), despite local coalmines, Scots, Austrian and other pines are represented, also spruce and larch.

The observations were extended to other classes of vegetation, especially agricultural crops; but these will be dealt with in a subsequent paper, along with the results of further investigations that are in progress.

It has been clearly demonstrated by several investigators that one great cause of the damage inflicted by smoke is the presence in it of sulphur dioxide. This penetrates the leaf and is retained there, so that in general leaves from smoke-polluted districts are richer in sulphur than similar leaves from clean districts. The sulphuric acid of the smoke will have much the same effect. Hence the sulphur-content of leaves should furnish guidance in the diagnosis of smoke-pollution, and indeed is now generally accepted as one of the most reliable aids to such diagnosis<sup>1</sup>.

In order to test this, samples of leaves (sycamore) were taken at the end of July 1911—after a month of dry weather—in each of four districts<sup>2</sup>—two dirty (S.W. 3 and S. 3) and two clean (N. 3 and N.E. 3). Each sample was carefully reduced to ash and the total

<sup>1</sup> Haselhoff and Lindau, *loc. cit.* 57.

<sup>2</sup> All on one geological formation, viz. the coal measures. The risk of complications arising from differences of soil was thereby probably reduced.

sulphate in the ash then determined (as  $\text{BaSO}_4$ ). The results are summarised below, together with the amounts of sulphur in the rain collected during the period July 1 to August 23 and the totals for twelve months (cf. p. 395).

District	$\text{SO}_3$ in dry matter* %	Total S. in rain July 1—Aug. 23 % $\text{SO}_3$	Total S. in rain (lbs. per acre per ann.) $\text{SO}_3$
S.W. 3	1.36	14.6	268
S. 3	1.11	13.3	269
N. 3	0.91	10.7	218
N.E. 3	0.55	10.0	186

In order to ascertain how much of the sulphate was on the outsides of the leaves, other samples were taken at S. 3 and N. 3 and dipped in distilled water six times before drying. The percentages of  $\text{SO}_3$  in the ash were then found to be reduced to .89 and .75 respectively—results, however, of only a single determination in each case.

In the following year the tests were repeated with greater precautions in the choice of samples and at different periods.

On May 29th, 1912, when leaf-blotching was first noticed, samples of 50 sycamore leaves were taken in each of four districts, the leaves being taken from a number of trees in each case. In one district (S. 3) duplicate samples were taken. In all cases leaves of about the same size were selected.

On August 13th, after a period of continuous heavy rain, another set of samples was taken. In each case determinations were made of the total sulphur, water-soluble sulphur, and ash in the leaves.

*Percentage in dry matter.*

District	Total $\text{SO}_3$		Water-sol. $\text{SO}_3$		Chlorine		Total ash		Condition of leaves	
	May	Aug.	May	Aug.	May	Aug.	May	Aug.	May	Aug.
N. 3 (clean)	.48	.75	.14	.48	.63*	.94*	6.8*	7.8*	Healthy	Slightly damaged
N.E. 3 (clean)	.54	.86	.29	.62	.63*	.91	4.9*	7.8	"	"
N.W. 3 (dirty)	.72	.83	.39	.69	.75	—	5.2*	8.0*	Damaged	) Had been practically killed for some time
S. 3 (very dirty) (1st sample)	.79	.86	.56	.80	—	—	5.3*	8.6*	"	
S. 3 (2nd sample)	.76	.85	.48	.83	—	—	—	—	"	

Single analysis only.

It will be noted that in the young leaves (May) the total sulphur showed a marked difference between the clean and dirty areas, but that the differences were appreciably less at the later stage of growth. The water-soluble (inorganic) sulphur, however, showed very pronounced differences at both stages.

It is commonly stated that smoke-damaged leaves contain a higher proportion of ash than normal leaves, and the data obtained in the cases quoted above support this view. They are too meagre, however, to serve as a basis for discussion, as are also the data for chlorine.

One further set of observations may be recorded since they bear out previous observations which have received some little criticism. In the previous paper the opinion, based upon microscopical examination of leaf-sections, was expressed that the solid matters of smoke might cause an actual clogging of the stomatal openings of leaves. Further examinations have recently been made by a different observer (D. W. S.) with leaves of evergreens taken on February 14th, 1913, after two days' dense fog. In each case a thin slice was skimmed off from the under surface of the leaf and subjected to microscopic examination. In the case of aucuba the soot occupied the stomatal openings on many preparations. Similarly with privet, the soot had gathered in the openings and also followed the lines of the guard cells. In the case of holly and ivy the soot was generally distributed, and showed no decided preference for the stomatal openings, although a few were occupied. The matter on the leaves and lodged in the stomatal openings where present was quite sharply defined and opaque.

#### SUMMARY.

The rain analyses summarised in this paper bring out very clearly the marked difference in the purity of the atmosphere between the industrial-rural area to the west and south and the purely agricultural area to the north and north-east of Leeds.

They show that the sulphur-content of the rain falling at a given station affords a fairly reliable diagnosis of the degree of pollution of the atmosphere by smoke, provided the observations be prolonged over several months. Further, evidence has been obtained in confirmation of that adduced by earlier observers, that the sulphur-content of the leaves of trees may afford useful assistance in the diagnosis of smoke-pollution. In preliminary tests the proportion of sulphur present in

the leaf as sulphate gave a sharper grading of the pollution in different districts than the total sulphur.

The rain analyses show further that appreciable smoke-pollution still remains throughout the agricultural area at distances of seven miles from the city, the rate of improvement of the atmosphere on passing away from the city into the purely agricultural areas being appreciably slower in the direction of the prevailing winds than in other directions.

In a general way the analyses tend to show that the smoke, though in greater quantity, is in a higher state of oxidation in the dirtier or more industrial districts—due to more efficient combustion of the coal.

Throughout the industrial-rural area abundant evidence of damage to leaves was found, and there can be no doubt of the consequent check to growth.

The investigations summarised in this paper have been rendered possible by a grant from the Board of Agriculture and Fisheries from funds placed at their disposal by the Development Commissioners, and we desire to express our appreciation of the opportunity thus afforded of carrying out the work which previous investigations indicated as being desirable.

We would also here acknowledge our indebtedness to all those who have granted us facilities for the collection of samples at the different centres, and to our colleague, Mr A. G. Ruston, B.A., B.Sc., for assistance in various ways.

Further experiments are in progress, by means of which we hope to get a direct measure of smoke damage upon agricultural crops in semi-urban areas, and also further criteria for its diagnosis.



## APPENDIX.

*Table showing range of variation in individual samples of rain, mean composition and probable error of mean for each station.*

(Parts per 100,000.)

	Total suspended matter	Ash in suspended matter	Chlorine	Total sulphur (expressed as SO <sub>3</sub> )	Total nitrogen
Station N. 7					
Max. ...	2.66	1.53	3.31	3.63	0.187
Min. ...	0.04	0.03	0.15	0.37	0.034
Mean ...	0.96 ± .12	0.51 ± .07	0.58 ± .13	1.70 ± .14	0.089 ± .007
Station N. 5					
Max. ...	3.50	2.38	5.84	6.08	0.235
Min. ...	0.36	0.10	0.25	0.50	0.037
Mean ...	1.88 ± .12	0.76 ± .10	0.79 ± .18	2.56 ± .23	0.121 ± .009
Station N. 3					
Max. ...	7.00	5.20	3.87	7.12	0.160
Min. ...	0.40	0.17	0.20	0.74	0.037
Mean ...	2.57 ± .23	1.57 ± .19	0.78 ± .11	3.19 ± 0.25	0.086 ± .006
Station N.E. 7 (c)					
Max. ...	4.76	3.09	3.57	5.87	0.234
Min. ...	0.32	0.07	0.15	0.69	0.031
Mean ...	1.79 ± .21	1.07 ± .14	0.65 ± .12	2.30 ± .22	0.091 ± .010
Station N.E. 5					
Max. ...	7.74	5.51	3.55	5.54	0.246
Min. ...	0.20	0.12	0.10	0.64	0.046
Mean ...	2.26 ± .35	1.39 ± .26	0.64 ± .13	2.52 ± .24	0.137 ± .010
Station N.E. 3					
Max. ...	3.97	2.50	2.63	6.62	0.364
Min. ...	0.20	0.06	0.30	0.67	0.055
Mean ...	1.73 ± .17	0.90 ± .12	0.65 ± .08	2.70 ± .27	0.123 ± .013
Station E. 7					
Max. ...	5.88	4.31	3.10	6.95	0.278
Min. ...	0.54	0.13	0.10	0.58	0.052
Mean ...	1.85 ± .25	0.95 ± .20	0.65 ± .10	2.54 ± .27	0.100 ± .008
Station E. 5					
Max. ...	11.13	4.47	3.82	7.16	0.302
Min. ...	0.68	0.42	0.10	0.74	0.027
Mean ...	3.26 ± .40	1.64 ± .20	0.76 ± .13	2.63 ± .23	0.123 ± .009
Station E. 3					
Max. ...	12.54	6.02	2.57	12.54	0.455
Min. ...	0.99	0.44	0.40	0.88	0.052
Mean ...	3.18 ± .41	1.73 ± .28	0.86 ± .09	3.29 ± .51	0.126 ± .020
Station S.E. 3					
Max. ...	22.82	17.47	3.30	12.85	0.492
Min. ...	1.03	0.74	0.30	2.16	0.049
Mean ...	6.04 ± .83	4.28 ± .69	1.08 ± .10	6.10 ± .45	0.152 ± .014
Station S. 3					
Max. ...	17.28	9.98	2.88	8.78	0.351
Min. ...	1.52	0.66	0.20	1.34	0.065
Mean ...	4.48 ± .51	2.42 ± .32	0.74 ± .10	4.22 ± .26	0.146 ± .009
Station S.W. 3					
Max. ...	6.77	5.11	2.85	8.59	0.193
Min. ...	0.65	0.57	0.27	0.74	0.053
Mean ...	3.23 ± .31	2.01 ± .22	0.75 ± .09	3.61 ± .34	0.107 ± .006
Station W. 3					
Max. ...	12.21	9.15	3.10	8.45	0.283
Min. ...	0.97	0.40	0.25	0.89	0.046
Mean ...	4.24 ± .44	2.59 ± .36	0.82 ± .08	4.13 ± .28	0.126 ± .009
Station N.W. 3					
Max. ...	7.79	5.12	2.25	12.39	0.354
Min. ...	0.90	0.46	0.30	1.65	0.050
Mean ...	3.03 ± .25	1.72 ± .17	1.09 ± .08	5.90 ± .46	0.116 ± .010

## CYANOGENESIS UNDER DIGESTIVE CONDITIONS.

BY S. J. M. AULD, D.Sc. (LOND.), PH.D., F.I.C.,

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THE considerable amount of attention paid, during recent years, to the liberation of hydrocyanic acid from plant products has caused grave suspicion to be cast upon certain of the latter which are commonly used for feeding purposes. Of the food-stuffs which produce hydrocyanic acid in considerable quantity on maceration with water, the most important are, without doubt, linseed and linseed cake, Java beans and other varieties of *Phaseolus lunatus*, and immature great millet (*Sorghum vulgare*). As is well known, definite cases of cattle-poisoning have been traced, in numerous instances, to the two last-named commodities, and linseed cake has also come in for a certain amount of suspicion, certain cases of poisoning amongst stock having been attributed to its cyanogenetic properties.

In all cases of prussic acid-forming feeding-stuffs so far examined the hydrocyanic acid is liberated by the hydrolysis of a glucoside by a co-existent enzyme, when the ground plant or seed is placed in water. The generally innocuous character of linseed cake was first explained by Henry and Auld<sup>(1)</sup> and by Dunstan<sup>(2)</sup> as being due to the destruction of the glucosidoclastic enzyme present in the seed during the hot process of expressing the oil. Further investigation by the author<sup>(3, 4)</sup> has shown that this view cannot be maintained, since the majority of linseed cakes produce hydrocyanic acid on maceration with water, the amounts varying from 0.001 per cent. to 0.052 per cent, and only in a few cases is no HCN formed, owing to the enzyme having been destroyed. On incubation at temperatures approximating to blood-heat half the available prussic acid is sometimes liberated in fifteen minutes and the maximum practically attained within six hours. Despite this fact, sheep fed with linseed cake containing 0.052 per cent.

of available HCN, in quantities up to 4 lbs. per day (5 lbs. per 100 lbs. live-weight), suffered no ill effects, although the proportion of available hydrocyanic acid far exceeded the lethal dose as determined by direct experiment with potassium cyanide (Auld, *loc. cit.*). Investigation has shown that the enzymic hydrolysis of the cyanogenetic glucoside is greatly inhibited by nearly all the conditions prevailing in the digestive tract. Acidity and alkalinity both have a strongly retarding influence on the rate of evolution of prussic acid, as also have the digestive enzymes themselves. Many of the feeding adjuncts have a strongly inhibiting action on cyanogenesis; salt, glucose and molasses are strongly active in this direction, as is also cellulose. The effect of the last named is most profound and was discovered by incubating ground linseed cake with many of the common fodders which are likely to be fed with it. Some of these fodders contain enzymes capable of hydrolysing sugars, glucosides, etc., but despite this fact the rate of evolution of hydrocyanic acid from linseed cake is invariably retarded thereby.

#### *Influence of Cellulose on Cyanogenesis.*

The fodders initially used in these experiments were mostly grown specially for the purpose, the green plants being dried at ordinary temperature, disintegrated and reduced to powder in the mill. In each case observations were made both with the powdered plant and with extracts prepared from it. 25 grams of cake were used in each experiment. The plant extracts were obtained by digesting the ground material with twelve volumes of water containing a few drops of toluene for 48 hours at 38 degrees C. The time of incubation was 30 minutes in each case.

The action of the various plant extracts, either in a positive or negative direction, is very small and is not to be correlated with the existence in them either of active enzymes (as in the case of rye, tares and maize) or of inhibiting substances like sugar. The inhibiting action of the ground plant on the other hand is considerable and invariable. That the effect is due to the cellulose was proved in the case of Helianti, a very coarse fodder. Fifteen grams of this material were ground and extracted successively with ether, alcohol and water. The various extracts and the exhausted residue were then incubated separately with 25 grams of linseed cake for 30 minutes at 35 degrees C.

TABLE I.

Plant	Weight of ground plant	Volume of extract	HCN formed	Temperature of action	Control HCN formed
	gram	c.c.	%	deg. C.	%
1. COMMON RYE. <i>Secale cereale</i> L. Just before flowering	{ — 15	50 —	0·032 0·022	38·0	0·030
2. COMMON VETCH OR TARES (Variety Winter). <i>Vicia sativa</i> , L. Cut in full flower	{ — 15	100 —	0·031 0·028	37·5	0·031
3. LUCERNE. <i>Medicago sativa</i> L. Flowers unopened	{ — 20	100 —	0·030 0·026	37·5	0·031
4. SAINFOIN (Variety Giant). <i>Onobrychis sativa</i> , Lam. In full bloom	{ — 15	100 —	0·029 0·025	37·5	0·031
5. KIDNEY VETCH. <i>Anthyllis vulneraria</i> , L. Height 10 inches	{ — 15	75 —	0·028 0·024	37·5	0·030
6. HELIANTH. <i>Helianthus macrophyllus</i> . Height 2 ft.	{ — 15 20	100 — —	0·018 0·013 0·011	38·0	0·026
7. MAIZE. <i>Zea Mais</i> . Height 4 inches	{ — 20	100 —	0·021 0·019	38·0	0·026
8. MAIZE. Height 30 inches .. .. .	{ — 15	100 —	0·021 0·017	38·0	0·026

Material used	HCN formed
Cake alone .....	0·021 per cent.
Cake + ether extract .....	0·019 " "
Cake + alcohol extract .....	0·017 " "
Cake + aqueous extract .....	0·019 " "
Cake + extracted residue.....	0·010 " "

Cellulose was prepared in a fine, disintegrated condition by digesting Swedish filter paper, cut into small pieces, successively with 2 per cent. sulphuric acid and 2 per cent. caustic soda. The addition, in this form, of cellulose to linseed cake depressed the formation of prussic acid in a similar manner to the fodders. A bulkier cellulose prepared from cotton wool had a similar effect. Experiments with salicin and amygdalin showed conclusively that this action is due to the adsorption of the cyanogenetic enzyme by the cellulose. As these experiments are being continued on other lines, they will not be quoted further here. It is sufficient to point out the profound inhibiting action of the fibrous feeding-stuffs on cyanogenesis from linseed cake under digestive conditions.

*Effect of Acids and Alkalies under Digestive Conditions.*

It is well known that most glucosidoclastic enzyme actions are strongly inhibited by dilute acids and alkalies, although mere traces of acid and base are occasionally helpful. In face of this knowledge, a statement by Lander<sup>(6)</sup> that "fermentation" (*i.e.* cyanogenesis) "goes on in 1 per cent. hydrochloric acid and also in 1 per cent. sodium bicarbonate solutions, and would not therefore be inhibited by the body fluids," appears remarkable, and is, as a matter of fact, incorrect. These concentrations are equivalent roughly to  $N/4$  HCl and  $N/12$   $NaHCO_3$ .

A communication by Collins<sup>(6)</sup> lays especial stress upon the effect of acidity, and points out that a linseed cake acidified to reproduce digestive conditions (about  $N/18$  HCl) fails to evolve hydrocyanic acid. Even acids of one-hundredth normal strength prevent the enzyme working, and hydrochloric acid of one-thousandth normal strength produces a slackening in the rate of evolution. The effect of acidity is certainly profound, though the author has found that one-hundredth normal hydrochloric acid may still allow a certain amount of cyanogenesis to take place under certain conditions. In the experiments described by Collins one gram only of linseed was used for each experiment (*loc. cit.* p. 100) and the HCN formed was continually removed by a current of inert gas. The experimental temperature used was 45 degrees C. which is above the optimum temperature of phaseolunata<sup>(7)</sup>. Working in closed vessels at 37.5 degrees C. with 20 grams of linseed cake for each experiment, the results quoted in Table II have been obtained.

Collins explains the generally innocuous character of linseed and linseed cake as due to this inhibiting action of acids. Indeed he says "Under normal circumstances, therefore, linseed cannot produce hydrocyanic acid when fed to carnivorous or herbivorous animals, because the acid present in the stomach prevents the enzyme in the linseed from acting," and later "Should a linseed, rich in cyanogenetic glucosides, be fed to a beast suffering from indigestion, of such a peculiar character that the food was not rendered acid, then prussic acid poisoning might set in." In making these statements Collins overlooks the general course of the food during intra-corporeal digestion. During mastication the food is mixed with the alkaline salivary juices before swallowing. In man the material is stored, without movement<sup>(8)</sup>, for a period probably of about half-an-hour, in the fundus or non-acid

secreting portion of the stomach. In ruminants the food is stored in the rumen for a still longer period, and I have traced the greater

TABLE II.

Strength of acid	Time of action	HCN formed	HCN formed	Extent of Cyanogenesis
	hours	gram	%	%
N/100	1.5	0.00039	0.0019	4.7
	17	0.00078	0.0039	9.7
N/200	0.5	0.00078	0.0039	9.7
	1	0.00104	0.0052	13.0
	6	0.00259	0.0129	32.2
	12	0.00281	0.0140	35.0
N/500	1	0.00142	0.0071	17.7
	3	0.00252	0.0126	31.5
	6	0.00363	0.0181	45.2
	12	0.00415	0.0207	51.7
Neutral	0.5	0.00160	0.0080	20.0
	1	0.00254	0.0127	31.7
	3	0.00437	0.0218	54.5
	6	0.00570	0.0280	70.0
	14	0.00777	0.0388	—

portion of a meal in the paunch two hours after feeding. The regurgitated and remasticated food then passes through the reticulum and omasum before it reaches the true acid secreting stomach. There is thus hardly any question of acid inhibition of the cyanogenesis, since

TABLE III.

20 grams of cake in 150 c.c. of solution for each experiment. Temp. = 37.5° C.

Strength of alkali	Time of action	HCN formed	HCN formed	Decomposition
	hours	gram.	%	%
N/100	1	0.00098	0.0049	12.2
	3	0.00168	0.0084	21.0
	6	0.00189	0.0094	23.5
	10	0.00220	0.0110	27.5
N/200	0.5	0.00067	0.0034	8.5
	1	0.00155	0.0077	19.2
	2	0.00186	0.0093	23.2
	5	0.00264	0.0132	33.0
	12	0.00310	0.0155	38.7
N/500	35 min.	0.00132	0.0066	16.5
	1 hour	0.00173	0.0086	21.5
	3	0.00285	0.0142	35.5
	5	0.00331	0.0165	41.2
	12	0.00427	0.0213	53.2

the contents of the paunch are almost invariably alkaline, and the honeycomb and manypplies generally alkaline or neutral.

What inhibition does take place is probably due to the alkaline saliva. The average alkalinity of human saliva as measured in a number of cases is N/60. That of ruminants is of the same order. The production of hydrocyanic acid from linseed is practically completely stopped by sodium hydroxide solution of this strength although, taking it altogether, alkali seems to be rather less active as an inhibitor than acid. (Table III.)

In order to trace the extent and orientation of cyanogenesis in the animal body, sheep were fed with linseed cake before being slaughtered, and the contents of the stomach examined. Through the kindness of Mr W. M. Colebrook of Reading, to whom the writer wishes to express his thanks, three Hampshire-Down tegs were set apart for this experiment and fed as follows:

No. 1. Given 1 lb. crushed linseed cake 2 hours before killing.

No. 2. Given 1 lb. crushed linseed cake and a few handfuls of hay 2 hours before killing.

No. 3. Given 1 lb. crushed linseed cake  $\frac{1}{2}$  hour before killing.

The linseed cake employed was the same as that used in the previous experiment (*vid. sup.*). After the animals were killed the stomachs were removed and the contents washed into large flasks and steam distilled. The distillates were made up to known volume, tested qualitatively for HCN by the Prussian blue test and the amount formed estimated by titration with iodine in the usual manner. In each case the paunch contents were alkaline towards litmus paper, and the two intermediate stomach compartments either neutral or faintly alkaline. In every case, also, by far the greater part of the meal was still in the rumen. In the case of sheep No. 3, this applied almost wholly. The amounts of hydrocyanic acid traced throughout are given in the following table:

TABLE IV.

Sheep	Hydrocyanic acid formed, gram					Percentage of "available" HCN actually formed
	Rumen	Reticulum	Omasum	Abomasum	Total	
No. 1	0.03056	0.00388	0.00142	0.00077	0.03663	20.1
No. 2	0.02357	0.00311	0.00160	0.00065	0.02893	15.9
No. 3	0.00906	Traces	—	—	0.00906	4.9

It may be gathered from the above figures that a certain amount of prussic acid generation will usually take place from cyanogenetic feeding-stuffs when eaten by animals; also that the greater part of this formation will take place in the paunch in the case of ruminants, and in the fundus portion of the stomach in other animals. The chief limiting factor would appear to be the alkalinity of the masticated food, since the acidity of the true stomach is sufficient completely to inactivise the enzyme. This is also assisted, secondarily, by the cellulose present in the ration, and by many of the other food components.

*Conditions of Possible Poisoning by Cyanogenetic Feeding-Stuffs.*

The nature and alkalinity of the saliva is known to vary considerably, but even in the conditions most favourable for cyanogenesis it is very doubtful whether the alkali concentration will fall sufficiently low to allow prussic acid to be formed in large enough quantity to produce toxic symptoms. Much more important is the possibility of feeding-stuffs being used which contain free acids or which may undergo acid fermentation in the animal's body. It is notorious that fresh grass is strongly acid, and Collins (*loc. cit.*) gives its average acidity as of the order N/20. Ensilage, root tops and many other foods are distinctly acid in character and the use of such materials is likely to neutralise the salivary alkalinity and render the rumen contents neutral or acid. In the former case the use of much linseed cake containing large quantities of available prussic acid may be fraught with danger. Fortuitous neutralisation of the masticated food in this way and the feeding of improperly made linseed gruel (Auld <sup>(4)</sup>) are probably responsible for all the cases of linseed poisoning which occur from time to time.

In this connection it is interesting to review the three principal cyanogenetic feeding-stuffs already enumerated. As shown above, linseed is likely to be dangerous only in certain circumstances. Immature sorghum is especially dangerous, yet it contains little more prussic acid than many linseed cakes. The reason for its toxic character is probably its natural acidity when fed in the green state. It is possible that if it were used as soilage and fed with ground chalk, in order partially to neutralise the acid, that young sorghum would be less dangerous.



The case of *Phaseolus lunatus* is rather different. On the theory elaborated above it should be governed by the same conditions as linseed cake, despite the fact that it contains a higher proportion of active enzyme. So many cases are known however of Java beans having caused poisoning after being boiled <sup>(9, 10)</sup>, whereby the enzyme is destroyed, that one is bound to reconsider the position with regard to this notoriously poisonous food-stuff. The author has shown (*loc. cit.*) that neither phaseolunatin nor amygdalin is hydrolysed in the animal's body, and the circumstances in general are so much against prussic acid formation that it seems possible that another and unsuspected poison may be present in the seeds of the varieties of *Phaseolus lunatus*. If this is so it would go a long way to clear up the question of cyanogenesis in feeding-stuffs, interesting and important alike to toxicologist and agriculturist.

The significance of prussic acid formation is also worthy of consideration from the point of view of the nutritionist. Small quantities are undoubtedly formed from certain food-stuffs in the animal's alimentary canal and it may be that this amount is distinctly beneficial. This view was originally broached in a private discussion with Dr Bernard Dyer several years back, but it is recent investigation only which has shown the likelihood of its being true. Prussic acid in small quantities is well known as a "tonic." Its formation to a limited extent in the case of linseed cake, which is inimitable for fattening purposes and for "finishing off," may be mere coincidence, but it is significant that hydrocyanic acid in other circumstances acts as a hormone, and it may possibly have a stimulating effect on the secretion of digestive juices or the activity of the lacteals and capillaries in the process of resorption. This is a point which may repay attention and the author is already following it up.

### *Summary and Conclusions.*

1. Under digestive conditions cyanogenesis is likely to be inhibited by acids and alkalies, digestive juices, cellulose, glucose, and molasses, salt and many other feeding-stuff constituents and adjuncts.
2. Owing to the time the food remains in the digestive tract before coming to the true stomach or the acid secreting portion of the stomach, normal inhibition is caused by the alkaline character of the salivary juices. This is likely to be the chief cause of the innocuous character of linseed cake.

3. In the case of sheep fed with linseed cake shortly before being killed small amounts of hydrocyanic acid were to be found, chiefly in the rumen.

4. Cyanogenetic feeding-stuffs are most likely to be poisonous when fed with acid containing or acid producing food-stuffs, or where the hydrocyanic acid is pre-formed, as in the case of an improperly made linseed gruel.

5. The small quantities of hydrocyanic acid normally produced from cyanogenetic feeding-stuffs may possibly have a strongly beneficial action.

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# ON OVARIOTOMY IN SOWS; WITH OBSERVATIONS ON THE MAMMARY GLANDS AND INTERNAL GENITAL ORGANS.

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## PART II.

IN a former paper<sup>1</sup> evidence was adduced that the black pigment frequently found in the mammary tissue of sows is not associated (at any rate directly) with the occurrence of heat. This evidence has since been supplemented by an examination of ten strips of bacon cut from the mammary region of ten different sows. These "belly pieces" were kindly sent to us by Messrs C. & T. Harris & Co. of Calne, to whom we are indebted. These pieces were taken in the ordinary course of business, in the belief that they were cut from sows on heat. All the strips were much discoloured by large quantities of black pigment, but only four of them showed any indication of increased vascularisation. These four were to some extent congested in the close neighbourhood of the mammary ducts, and were probably obtained from sows that were on heat at the time of killing; the other six showed no such indications.

That the existence of black pigment in the mammary region is not related (at any rate directly) to the occurrence of heat is further proved by observations upon young Berkshire pigs, which had never been in use. In eight of these, four of which were spayed, and four "open," mammary pigment in some abundance was found to occur. In four others, however (two "open" and two spayed), no pigment was found excepting for a minute trace in one.

Another Berkshire sow was killed in young, and one of the foetuses was preserved by Mr John Hammond, Government Research Scholar

<sup>1</sup> *This Journal*, vol. iv. June 1912.

under the Board of Agriculture, who made histological preparations from the mammary region. Mr Hammond has kindly written for us the following account of his observations on this foetus, together with a note concerning his examination of a 7 weeks old pigling :

"The foetus in question (being out of a Berkshire sow showing no black pigment in her mammary glands) was examined for pigment. Sections were cut through the mammary region of the skin, and definite melanin granules were found in the rete mucosum. Further, the granules could be traced in places where the mucosum was seen dipping down to form the glands. In Fig. 1 the particles are shown to be following the course of the milk ducts, where at this stage they may be observed penetrating the dermis.

"Sections were also cut from the glands of a 'Large-Black' sow 7 weeks old. These show the pigment granules in the lower layers of cells in the rete mucosum of the skin, the colour being more intense at the bottom of the papillae. The hair follicles also showed black granules, being more intensely pigmented than the skin. In this case again the pigment granules could be seen following the course of the ducts from the nipple downwards to the gland. In the cells of the milk ducts the pigmentation was not so intense as in the cells of the glandular portion. These facts suggest that the pigment is a product of the metabolic activity of these cells, and that it is formed in greatest quantities where active growth is taking place.

"It is possible that the cells containing these granules increase at puberty and during pregnancy, or at such other times as the mammary gland hypertrophies, and so give rise to the pigmented condition which is seen in many cases over large areas in the mammary region of the adult sow."

If Mr Hammond's suggestion should prove to be correct, it provides an additional reason for spaying to those already put forward. This is a matter now under investigation.

It is interesting to note that whereas the foetuses (or at any rate one of them) contained mammary pigment, none was to be found in the pregnant mother. The non-occurrence of pigment in the mammary tissue either in this sow or in the piglings referred to above, indicates the possibility of breeding strains of Berkshire or of other coloured pigs in which there would be no melanin pigment in the bacon cut from the mammary region. The manner in which the presence or absence of this pigment is inherited is a point which requires investigation.

*Imperfect Spaying.*

In our former paper we gave expression to the suspicion that faulty operating was not altogether uncommon in the ordinary process of spaying sows for commercial purposes. This opinion we based largely on the fact, so frequently asserted, that some spayed sows behaved as though they were "open." We are now in a position to give definite information upon this point.

Through the courtesy of Mr Morris Wright of Whitton, Ipswich, who buys largely in the Ipswich district, two young sows were procured for us. They were both said to have been operated upon, but nevertheless were found to be behaving as if "open." These two sows (A and B) were under observation at the University farm for five and eight weeks respectively, and were found to behave as follows:

Case A showed signs of typical oestrus recurring at the normal intervals. At one of these periods this sow was put with the boar, and after considerable trouble, though possibly not more so than often happens with a normal sow mated for the first time, copulation took place. Six days after this service the sow was slaughtered and her viscera examined. The left ovary, the left Fallopian tube and the left half of the uterus were found to be present normally developed (Fig. 4). Numerous Graafian follicles in various stages of maturation were seen in the ovary, while the horn of the uterus had obviously undergone great hypertrophy since the date of the operation. No trace was found either of the right ovary, tube or uterine horn, which had evidently been removed completely. The position of junction of the right horn with the left was indicated by a scar, and there was a trace of the original wound made at the time of operation on the outside skin of the body.

Case B. Oestrus in this case was much less definite than in the former. Though the general appearance was unmistakable, swelling and congestion were less pronounced, the period was much lengthened, and there seemed to be no certainty about the date of recurrence. Notwithstanding that she was put with the boar on several consecutive days on which she showed signs of heat, in fact every day until all signs had disappeared, she resolutely refused his attentions. Some days after visiting the boar this sow was slaughtered and her viscera examined. The uterus was entirely absent. The right ovary also had evidently been completely extirpated along with the right Fallopian tube, but the left ovary with ripening follicles and large cysts (possibly formed from follicles) was found to be present (Fig. 5). The left Fallopian tube was absent.

The condition found in Case A is probably explained in view of the method of carrying out the operation. We reproduce, through the kindness of Mr J. G. Runciman, M.R.C.V.S., in Fig. 6 the sketch of a uterus which was torn in such a way as, in the hands of an unskilful operator, would have led to the retention of one horn of the uterus together with the corresponding ovary. As the occurrence of imperfect spaying is a matter of no inconsiderable importance from the economic standpoint, it is perhaps pertinent to give an account of the operation, and describe how this accident is liable to take place.

Ovariectomy on the pigling is performed in two positions. In one case the sow is laid flat on her right side with the left hind leg drawn slightly back; in this position the animal is held by an assistant. In the other case the operator, standing upright, swings the young sow by the left leg, puts his right foot on the left side of the neck and slightly arches the body of the sow by bending it across his left leg, thus bringing the left side of the abdomen rather more forward; the surgeon leaning forward to operate. In both cases the drawing back of the left hind leg stretches the flank and abdominal muscles on the left side. The next part of the operation consists in shaving the hair off a small area of skin situated close up to the loin, immediately in front of the thigh. It is in this area that the stab is made through the abdominal wall, admitting the index finger and the withdrawal of the "bed." The stab is made close up to the *loin* about half an inch in front of the ilium. The abdominal wall is pierced by means of a one-edged bistoury in a vertical direction. The blunt edge of the knife is uppermost when the stab is made, or in other words, the back of the blade is against the lumbar muscle. The stab when made by a good operator is only sufficiently extensive to admit of the introduction of one finger into the abdominal cavity. The finger is introduced directly after this incision is made, and the "bed" (*i.e.* the uterus together with the Fallopian tubes) secured by a hooking movement of the finger. Sometimes the middle of one horn of the uterus is all that is at first secured; under delicate manipulation this, in the form of a small contorted, worm-like tube, is generally drawn out through the incision, the rest of the uterus following. It is to be remarked that normally the infantile ovaries remain attached to the horns of the uterus by means of the broad ligament, and so are withdrawn from the body cavity along with them. Occasionally, more especially if the manipulation is not very carefully done, the traction which is needed to draw out the horn of the uterus first seized is so great as to lead to its tearing off, leaving

the rest of the organ behind in the body cavity. This appears to be the way in which imperfectly de-sexed animals are produced in the hands of careless or ignorant operators. Under such circumstances as those described the proper course is to begin again, reintroduce the finger, regrasp the uterus, a matter often of no small difficulty, and extract whatever remains of the organs referred to. The whole internal generative tract beyond the vagina should now be excised. It should be remarked that the division ought to be made externally, i.e. the knife is not to be introduced into the body cavity. The tear above referred to should be avoided, for owing to the position of the right uterine horn in the body cavity it is very difficult to reach it with the finger when once it has been severed from the left horn.

Though it so happens in the case illustrated (Fig. 6) that the right horn was the first part removed, Mr Runciman tells us that in his experience the left is usually the one likely to be torn away through this accident. The traction on the organs should be very gentle till the bulk of the uterus is secured; in fact it is safer when only one horn has been secured to draw it between the index and second fingers rather than between the index and the thumb.

#### *Summary of Practical Conclusions.*

Black pigment is very frequently, but not invariably present in the mammary tissue of Large-Black, Berkshire and other dark-coloured sows. It is stated that the presence of this pigment (apart altogether from the question of the pigs being "on heat") renders the bacon less saleable, owing to its discoloured or "seedy" appearance<sup>1</sup>. If this is the case, it ought to be possible to remedy this fault, by breeding from individuals in which this pigment is absent, and so building up a strain of increased commercial value.

If on the other hand it be the case that the presence of black pigment is not in itself objectionable, but that the changes in the mammary area due to the occurrence of heat are what are not desired, the remedy is to be sought in spaying, a practice which must be carried out on a sufficient scale and be efficiently performed. That imperfect spaying (in which one, or a part of one, ovary is left behind, while the bed is wholly or partly removed) sometimes happens is shown by the facts described in this paper.

<sup>1</sup> This matter has now (July 9th, 1913) been further investigated and will be treated of again in a future paper.







Fig. 1.



Fig. 2.



Fig. 3.



Fig. 4.



Fig. 5.



Fig. 6.

The expenses of the investigation described in this paper have been very largely defrayed by a grant made by the Development Commissioners through the Board of Agriculture and Fisheries.

#### DESCRIPTION OF PLATE.

FIG. 1. Section through developing mammary tissue of foetal pig showing pigment granules in the rete mucosum and in places where this layer is seen dipping inwards to form the glands.

FIG. 2. Transverse section through duct of nipple of Large-Black sow showing pigment in the epithelial cells.

FIG. 3. Longitudinal section through same.

FIG. 4. Left ovary, tube, and horn of uterus found remaining in sow which was supposed to have been spayed (Case A in text). Much reduced.

FIG. 5. Left ovary with large cysts found in sow which was supposed to have been spayed (Case B in text). Reduced.

FIG. 6. The two horns of a uterus which had been accidentally almost completely severed in the operation of spaying (A, the point where the tear had been made. See text).

## THE COMPOSITION OF IRRIGATED AND NON-IRRIGATED APPLES.

BY J. S. JONES AND C. W. COLVER.

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EXPRESSION is frequently given to an apparently widespread belief that irrigated in comparison with non-irrigated fruits are flat in taste and less resistant to the various agencies which effect decay. In support of that belief this statement in substance is almost invariably advanced: "The irrigated fruits contain abnormally high percentages of water and consequently low percentages of solid or dry matter; they are, therefore, deficient in the particular compounds upon which taste and body or solidity of structure depend." We do not presume to say that from analyses alone can the many questions relating to quality in fruits be definitely settled, but since those alleged characteristics of irrigated fruits are charged by this statement to radical deficiencies in certain compounds, it would seem that analytical data would be of material service in the settlement of questions relating to quality. This view of the matter and the fact that here in the north-west, in both the irrigated and the non-irrigated sections, the hardy fruits are grown extensively and shipped to distant markets induced us to undertake some two years ago extensive analyses of fruits grown with and without irrigation. We wish to report here in summarised form the results of that work on the apple; for it, in point of commercial importance, stands pre-eminent among all other fruits grown in the north-west.

It should be particularly noted that none of the samples were secured from especially controlled conditions; all irrigated samples were grown in sections where climate and soil render irrigation imperative, all non-irrigated samples in sections where the annual precipitation varies from 25 to 35 inches and where the soil and

topography of the country render irrigation methods impracticable. The analyses, therefore, indicate the composition of *normal* irrigated and *normal* non-irrigated apples.

For the benefit of those who might wish to compare these analyses with similar ones from other sources, a brief statement will be made in reference to methods of analysis adopted at the beginning of the work and closely adhered to throughout. The determinations were limited to those constituents which are believed to be of greatest influence on taste and other qualities and at the same time subject to widest variation because of environmental or cultural conditions. In all cases the whole fruit exclusive of the stem, seeds, and smallest possible core was used for the analysis.

*Total solids* were determined by the combined use of the ordinary drying oven and the vacuum desiccator. Portions of the fruit, cut into very small cubes, were kept at a temperature of 40 to 45 degrees Centigrade in the drying oven for approximately 24 hours. They were then removed to the vacuum desiccator where drying was continued to practically constant weight in a vacuum of 20—22 inches and with concentrated  $H_2SO_4$  as the drying agent.

*Acids* and *sugars* were determined in aliquot portions of the extract obtained by digesting a weighed portion of the finely chopped fruit (twice the normal weight for the Schmidt and Haensch polariscope) for several hours with successive portions of warm water. The acids were titrated with N/10 NaOH and calculated as  $H_2SO_4$ . *Invert* and *cane* sugar were determined by precipitation of cuprous oxide from Fehling's solution as modified by Munson and Walker. For the conversion of weights of cuprous oxide to corresponding weights of invert sugar, Munson and Walker's tables were used. *Insoluble solids* were determined by drying at 100 degrees Centigrade to constant weight the residue from the portion digested for acids and sugars.

Nitrogen was determined by the Kjeldahl method on 10—12 gram portions of the fruit; the *crude protein* by multiplying the nitrogen percentage by 6.25.

From raw ash obtained by burning at low red heat in a muffle furnace (leaching and reburning when necessary), *pure ash* was calculated by subtraction of unconsumed carbon, carbon dioxide, sand, and soluble silica.

For the determination of waste, mechanical methods of necessity were resorted to. Although they, in comparison with chemical methods, are incapable of the same degree of accuracy, it should be noted that

TABLE I. Summary of Analyses of Irrigated and Non-Irrigated Apples.

Variety and how grown	Number of analyses averaged	Water per cent.	Solids		Sugar		Acids as H <sub>2</sub> SO <sub>4</sub> N x 64 per cent.	Crude pro-tein N x 64 per cent.	Pure ash per cent.	Edible		Waste	
			Total per cent.	In-soluble per cent.	Invert per cent.	Cane per cent.	Total per cent.					Skins per cent.	Core per cent.
Aiken Red, irr. ....	3	83.02	16.98	2.76	8.01	2.81	10.82	.233	.175	90.99	3.71	5.30	9.01
Arkansas Black, irr. ....	5	81.59	18.41	3.02	7.58	4.04	11.62	.344	.245	91.49	2.75	5.76	8.51
" " non-irr. ....	6	81.64	18.36	3.64	7.40	4.86	12.26	.306	.361	90.02	3.95	6.03	9.98
Ben Davis, irr. ....	7	83.64	16.36	3.30	6.48	3.58	10.06	.283	.179	88.33	5.01	6.66	11.67
" " non-irr. ....	17	81.31	18.69	4.27	7.58	3.42	11.00	.288	.334	85.23	8.45	6.32	14.77
Gano, irr. ....	3	83.98	16.02	2.92	6.51	3.67	10.18	.316	.150	86.27	7.32	7.43	13.73
" non-irr. ....	21	82.48	17.52	3.58	7.09	3.72	10.81	.264	.293	87.97	7.43	5.60	13.03
Grimes Golden, irr. ....	1	79.89	20.11	2.70	6.15	6.10	12.25	.322	.194	84.95	5.10	9.95	15.05
" non-irr. ....	4	82.91	17.09	3.09	5.87	5.29	11.16	.394	.247	89.54	5.91	4.55	10.46
Jonathan, irr. ....	33	82.67	17.33	2.42	7.80	3.30	11.10	.398	.204	88.53	6.78	4.69	11.47
" non-irr. ....	46	82.61	17.39	2.87	7.80	3.20	11.00	.403	.250	87.38	7.03	5.59	12.62
Kinnard, irr. ....	3	84.02	15.98	2.73	8.72	1.55	10.27	.296	.194	90.12	6.03	3.25	9.88
Mammoth Black Twig, irr. ....	8	82.10	17.90	2.86	8.20	3.54	11.74	.318	.188	90.12	6.06	4.10	10.16
Rhode Island Greening, non-irr. ....	1	82.80	17.20	2.94	6.61	3.44	10.05	.430	.470	79.31	—	—	20.69
Rome Beauty, irr. ....	25	84.81	15.19	2.40	5.88	4.12	10.00	.249	.195	90.01	6.04	3.95	9.99
" non-irr. ....	33	83.08	16.92	2.98	6.56	4.06	10.62	.308	.266	88.09	6.26	3.74	10.00
Spitzenberg, non-irr. ....	8	82.31	17.69	3.56	6.47	4.03	10.60	.353	.320	88.09	7.26	4.65	11.91
Tompkins King, non-irr. ....	6	81.51	18.49	2.47	6.75	4.98	11.73	.366	.336	91.38	5.73	2.89	8.62
Wagener, non-irr. ....	13	84.98	15.02	2.51	5.46	4.34	9.80	.287	.255	90.13	5.98	3.89	9.87
White Pearmain, irr. ....	3	82.74	17.26	3.10	7.24	3.84	11.08	.222	.205	87.42	7.32	5.26	12.59
Wineap, irr. ....	7	80.63	19.37	2.71	10.40	2.10	12.50	.285	.203	88.75	6.59	4.66	11.25
Winter Banana, irr. ....	6	83.59	16.41	2.70	6.09	3.63	9.72	.366	.248	89.48	7.36	3.16	10.52
" non-irr. ....	9	83.25	16.75	2.93	6.65	3.61	10.26	.360	.259	90.08	6.58	3.39	9.97
York Imperial, irr. ....	8	83.48	16.52	3.02	7.14	3.79	10.93	.301	.194	91.31	5.43	3.21	8.69
Yellow Newtown, irr. ....	4	82.71	17.29	2.92	7.68	3.72	11.40	.387	.177	90.76	5.89	3.35	9.24
" non-irr. ....	5	80.43	19.52	4.12	6.96	4.61	11.60	.469	.378	—	—	—	—
All Varieties, irr. ....	116	83.14	16.86	2.66	7.31	3.53	10.84	.322	.200	89.40	6.43	4.18	10.60
" " non-irr. ....	168	82.61	17.39	3.19	7.02	3.82	10.84	.336	.283	88.26	6.88	4.81	11.74

\* One determination.

the determinations were invariably made by the same analyst. The personal equation was thus eliminated.

In all cases the samples were harvested when the owners of the several orchards in which they grew were harvesting the varieties they represent for market purposes. Moreover all samples were held in storage previous to analysis so as to reach the analyst at approximately the same time they would have otherwise reached the retail trade.

Represented in Table I are the most prominent commercial varieties of Idaho. Possibly other varieties are more prominent in other sections, but these are well known throughout the north-west.

Of a majority of those varieties, eight in all, which were found growing in *both* irrigated and non-irrigated sections, the average irrigated sample contained the smaller percentage of total solids, total sugar, and acid; of all those varieties it contained the smaller percentage of insoluble solids and crude protein. If, in the final summing up, variety distinctions are lost sight of and the classification of samples is made solely on the basis of irrigation or non-irrigation, the average irrigated sample contained the same percentage of total sugar, but smaller percentages of each of the other constituents.

But upon whatever basis comparison of composition is made differences between the irrigated and the non-irrigated in total sugar and acid are so small that we feel justified in the conclusion that there is in the analytical data no substantial basis for the claim that the irrigated apple is inferior in taste. Its uniformly lower content of solids insoluble in water may lend some support to the belief that it is slightly inferior in keeping properties.

When average content of insoluble solids, total sugar, acid, and crude protein were calculated on average content of total solids or dry matter the results shown in Table II were obtained.

Of a majority of those varieties which are represented by both irrigated and non-irrigated samples, the dry matter of the average irrigated sample contained the greater percentage of total sugar; of one-half of those varieties it contained the greater percentage of acid; of all of those varieties it contained the smaller percentage of insoluble solids and crude protein. If variety distinctions are again lost sight of, and, as before, the classification of samples is made solely on the basis of irrigation or non-irrigation, the dry matter from the average irrigated sample contained the greater percentage of total sugar, practically the same percentage of acid, but smaller percentages of insoluble solids and crude protein.

*Composition of Apples*TABLE II. *Insoluble Solids, Sugar, Acid, and Crude Protein calculated on Average Content of Dry Matter.*

Variety and how grown	Number of analyses averaged	Insoluble solids per cent.	Sugar total per cent.	Acids as H <sub>2</sub> SO <sub>4</sub> per cent.	Crude protein N x 6½ per cent.
Aiken Red, irr. ....	3	16.24	63.66	1.37	1.03
Arkansas Black, irr. ....	5	16.41	63.06	1.86	1.33
" " non-irr. ....	6	19.33	66.78	1.67	1.97
Ben Davis, irr. ....	7	20.17	61.50	1.73	1.09
" " non-irr. ....	17	22.85	58.85	1.54	1.78
Gano, irr. ....	3	18.23	63.55	1.97	.94
" " non-irr. ....	21	20.43	61.70	1.50	1.67
Grimes Golden, irr. ....	1	13.43	60.92	1.60	.96
" " non-irr. ....	4	18.08	65.30	2.30	1.44
Jonathan, irr. ....	33	13.97	64.05	2.30	1.17
" " non-irr. ....	46	16.51	63.25	2.32	1.43
Kinnard, irr. ....	3	17.08	64.26	1.85	1.21
Mammoth Black Twig, irr. ....	8	15.92	65.59	1.78	1.05
Rhode Island Greening, non-irr. ....	1	17.09	58.43	2.50	2.73
Rome Beauty, irr. ....	25	15.80	65.83	1.64	1.28
" " non-irr. ....	33	17.61	62.77	1.82	1.57
Spitzenberg, non-irr. ....	8	20.13	59.36	2.00	1.80
Tompkins King, non-irr. ....	6	13.36	63.44	1.98	1.81
Wagener, non-irr. ....	12	16.71	65.25	1.91	1.70
White Pearmain, irr. ....	3	17.96	64.14	1.29	1.19
Winesap, irr. ....	7	13.99	64.53	1.47	1.05
Winter Banana, irr. ....	6	16.45	59.23	2.35	1.51
" " non-irr. ....	9	17.49	61.25	1.73	1.54
Yellow Newtown, irr. ....	4	16.89	65.93	2.24	1.02
" " non-irr. ....	5	21.11	59.43	2.40	1.93
York Imperial, irr. ....	8	18.28	66.16	1.82	1.17
All Varieties, irr. ....	116	15.78	64.30	1.91	1.18
" " non-irr. ....	168	18.34	62.33	1.93	1.62

By force of circumstances in the north-west as elsewhere the desiccated apple must be looked upon as an article of growing commercial importance. Differences in composition of dry matter which have just been noted are small; if based on the commercial article they would seem smaller. Therefore as between the desiccated product from the irrigated and that from the non-irrigated orchard there appears to be no substantial basis for market discrimination.

## DIGESTIBILITY EXPERIMENTS WITH SHEEP. PARA RUBBER SEED CAKE.

By S. J. M. AULD, D.Sc., Ph.D., F.I.C.,

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THE experimental determination of the digestibility of feeding stuffs has been carried out only to a very limited extent in this country. Nearly all the tables of digestibility-coefficients are of Continental origin, those of Kellner having pre-eminence and being generally adopted. The desirability of carrying out digestibility trials with home-grown feeding stuffs and with new feeding materials obtained from the Colonies and India is, however, apparent, and this is also the case with many of the proprietary feeds which are much used in this country. Tables built up on these lines would materially assist in the calculation of rations, this being at present frequently a matter of difficulty owing to the necessity of estimating an average digestibility, for example, for bye-products incorrectly named or differing slightly from those generally quoted, for materials which may possibly differ considerably in digestibility when grown under different conditions and for the numerous proprietary articles such as the molasses feeds (cf. Goodwin, *Journal Board of Agriculture*, 1911, 18, 97). A mere extension of the digestibility coefficients already tabulated would be of extreme value, but a judicious replacement or confirmation of the Continental figures for home-grown materials would seem to be still more useful.

Experiments with sheep on these lines were instituted at Wye College and the author intends to continue them at Reading. The trials were carried out with sheep and the results obtained with Para rubber seed cake are offered herewith. The *modus operandi* was essentially that generally adopted and was, in fact, elaborated by Goodwin after personal investigation of Kellner's methods.

As the trials proceeded several modifications were made in the apparatus, particularly with regard to the sheep harness, which is



rather difficult to maintain in position over an extended period. This was largely overcome by making all the connections of broad "elastic," fitted with flat buckles, which allowed any necessary adjustment from time to time. Three sheep (Kents) were used in each investigation, in order to provide sufficient control if any one animal became disordered. Throughout the experiment the boxes were placed in such a manner that each sheep could see every other sheep, since this greatly benefits their docility.

*Para Rubber Seed Cake.*

This product is the press-cake left after expression of the oil from the kernels of the seed of the Para rubber tree (*Hevea brasiliensis*, Müller Arg.). Enormous quantities of these seeds are available every year and the quantity is certain to increase (cf. *Bull. Imp. Inst.* 1903, 156). Their economic importance would appear to be considerable as they contain 42—49 per cent. of a pale yellow drying oil which has been investigated by Pickles and Hayworth (*Analyst*, 1911, 36, 493). There is also present a lipoclastic enzyme and a cyanogenetic glucoside (Dunstan, *Proc. Chem. Soc.* 1907, 168). The seeds themselves yield about 0.048 per cent. of prussic acid (Henry and Auld, *Journ. Soc. Chem. Ind.* 1908, 27, 428). In analogy with linseed cake it appeared therefore, *prima facie*, that the resultant press-cake might be dangerous for use as a cattle food owing to its high content of HCN, which, by calculation, would be expected to be of the order 0.09 per cent., although hot pressing might partially destroy the glucosidoclastic enzyme.

The material used in this investigation was supplied by Professor W. R. Dunstan, F.R.S., Director of the Imperial Institute. It was a light brown very friable cake of rather peculiar, but pleasant smell, and despite the ease with which it crumbled, was found to contain about 20 per cent. of "oil." The cake was examined for prussic acid by the usual methods but none could be detected, either as cyanogenetic glucoside or in the "free" state. This is remarkable and argues a different condition of affairs from those existing in linseed.

Animals given the cake took to it readily and no great difficulty was experienced in this way with the experimental sheep, even when fed over an extended period.

The basal ration adopted in the trials consisted of linseed cake and hay, and the periods adopted were of about one week in duration throughout, viz. (1) seven days basal ration of 300 grams chaffed hay

and 150 grams linseed cake, fed thrice daily, without collecting faeces; (2) the same, faeces being collected; (3) eight days experimental ration (basal ration + 150 grams Para cake); (4) the same, faeces being collected. The sheep No. 3 showed signs of finishing its experimental ration only with difficulty and throughout the fourth period was given an extra 30 grams of a proprietary condimental food at each meal.

The results obtained were as follows:

*Analysis of Para Rubber Seed Cake.*

Moisture ... ..	9.27 per cent.
Crude protein ... ..	29.84 "
Crude fibre ... ..	3.15 "
Ether extract ... ..	20.11 "
Nitrogen-free extractive matter ... ..	33.08 "
* Ash ... ..	4.55 "
* Containing sand .. ..	0.23 "

*Analysis of Basal Ration Products.*

	Hay	Linseed cake	Condimental food
Moisture ... ..	9.70	14.00	11.12
Crude protein ... ..	8.97	28.43	11.32
Crude fibre ... ..	25.26	11.60	2.64
Ether extract ... ..	1.82	7.80	3.36
Nitrogen-free extractive matter ... ..	48.33	31.15	66.33
* Ash ... ..	6.42	7.02	5.23
* Containing sand ... ..	1.27	1.48	0.29

The moisture in the hay before grinding was 14.03 per cent. and the analytical figures were calculated back to this basis.

*Analysis of Faeces.*

	Basal ration			Experimental ration		
	No. 1	No. 2	No. 3	No. 1	No. 2	No. 3
Moisture ... ..	12.38	10.33	11.23	9.80	9.89	10.81
Crude protein ... ..	9.46	9.02	9.03	10.44	10.45	9.88
Crude fibre ... ..	32.40	35.12	32.72	34.00	33.80	30.85
Ether extract ... ..	1.09	1.44	1.47	1.49	1.79	1.79
Nitrogen-free extractive matter	32.33	33.86	35.35	34.90	34.70	34.00
* Ash ... ..	12.34	10.23	10.20	9.37	10.60	10.67
* Containing sand ... ..	2.66	2.72	2.38	2.56	2.64	2.55

*Sheep No. 1.*

*Basal ration.*—900 grams chaffed hay,  
450 grams linseed cake per day.  
Average 720 grams dung per day.

	Moisture	Ether extract	Crude fibre	Crude protein	N-free extract	Ash
Hay ... ..	126.27	11.25	216.90	76.86	414.09	54.99
Linseed cake ... ..	63.00	35.10	52.20	127.92	140.01	31.59
	189.27	46.35	269.10	204.78	554.10	86.58

*Digestibility Experiments with Sheep*

Dung ... ..	81.90	7.84	232.77	67.74	232.56	74.16
Basal ration digested ...	107.87	38.51	36.83	187.04	321.54	12.42
Coefficient of digestibility		88.07	13.50	66.93	55.05	

*Experimental ration.*—900 grams chaffed hay,  
450 grams linseed cake,  
450 grams Para rubber seed cake per day.  
Average 680 grams dung per day.

	Moisture	Ether extract	Crude fibre	Crude protein	N-free extract	Ash
Hay ... ..	126.27	11.25	216.90	76.86	414.09	54.99
Linseed cake ... ..	68.00	35.10	52.20	127.92	140.01	31.59
Para cake ... ..	41.71	90.49	14.14	134.28	148.86	20.47
	230.98	136.84	283.24	339.06	702.96	107.05
Dung ... ..	66.64	10.13	231.20	70.99	237.32	72.39
Experimental ration } digested ... ..		126.71	52.04	268.07	465.64	
Para cake digested ... ..		88.20	15.71	131.03	144.14	
Coefficient of digestibility } of Para cake		97.4	100*	97.5	96.7	

\* Containing sand.

*Sheep No. 2.*

*Basal ration.*—Average 630 grams dung per day.

	Moisture	Ether extract	Crude fibre	Crude protein	N-free extract	Ash
Dung ... ..	70.11	9.06	221.25	56.70	213.31	64.47
Basal ration digested ...	119.16	37.29	47.85	148.08	340.78	22.11
Coefficient of digestibility		80.45	17.77	72.32	61.45	

*Experimental ration.*—Average 650 grams dung per day.

	Moisture	Ether extract	Crude fibre	Crude protein	N-free extract	Ash
Dung ... ..	64.81	11.71	218.28	68.49	224.17	71.25
Experimental ration digested		125.13	64.96	270.57	478.79	
Para cake digested ... ..		87.84	17.11	122.49	138.01	
Coefficient of digestibility } of Para cake		97.1	100*	91.2	92.7	

\* Containing sand.

*Sheep No. 3.*

*Basal ration.*—Average 630 grams dung per day.

	Moisture	Ether extract	Crude fibre	Crude protein	N-free extract	Ash
Dung ... ..	70.74	9.25	206.01	56.89	222.70	64.26
Basal ration digested ...		37.10	63.09	147.89	331.40	
Coefficient of digestibility		80.0	28.4	72.2	59.8	

*Experimental ration.*—Ration + 90 grams condimental food per day.  
Average 675 grams dung per day.

	Moisture	Ether extract	Crude fibre	Crude protein	N-free extract	Ash
Ration ... ..	230.98	136.84	283.24	339.06	702.96	107.05
Condimental food ... ..	10.00	3.03	2.37	10.18	59.68	4.71
	240.98	139.87	285.61	349.24	762.64	111.76

Dung ... ..	72.96	12.07	202.50	81.67	229.50	78.00
Experimental ration } digested		127.80	88.11	267.57	538.14	
Para cake+condiment } digested		90.70	20.02	119.69	201.74	
Condiment digested ...		2.58	1.33	7.12	55.15	
Para cake digested ...		88.12	18.69	112.57	146.59	
Coefficient of digesti- } bility of Para cake		97.3	100*	84.0	96.7	
Average coefficient of } digestibility of Para } rubber seed cake		<u>97.2</u>	<u>100</u>	<u>90.09</u>	<u>95.3</u>	

\* Containing sand.

The experiments give results which are fairly concordant, sheep No. 3 showing the greatest divergence from the average. It is noteworthy that this was the animal which received the condimental addition to its ration and this may have had some disturbing influence. In correcting for the amount of the proprietary feed added, the digestibility of its protein content was estimated by treatment with pepsin-hydrochloric acid and was found to be 71 per cent. A microscopical examination showed the food to be composed of about 70 per cent. maize, 10 per cent. crushed beans, about 10 per cent. of desiccated cocoa-nut and the remainder apparently of spices; fenugreek in particular was detected by its smell. It was on this basis that the digestibility coefficients of the proprietary condiment were estimated.

In each case the crude fibre of the Para rubber seed cake shows a digestibility coefficient actually greater than 100 per cent. The reason for this is not very obvious. Possibly the Para cake offered a better medium for bacterial growth, or it may itself contain a cellulose-splitting enzyme. The excess is not, however, very large and there seems little doubt that practically the whole of the small amount of fibre of the rubber cake is digested.

The figures obtained show the Para cake to be one of the most digestible concentrated foods available. This is no doubt partly due to the small amount of crude fibre present. This, and the absence of mucilage as in linseed cake, means a lack of "binding" material and probably accounts for the extreme friability of the product.

The results are offered without prejudice to future work on "digestibility" of food stuffs or to the value ascribed by the author to the present accepted figures.

The author's thanks are due to Mr F. Knowles and Mr T. D. Moss crop, B.Sc., for their help in carrying out this particular trial and, in particular, to Mr R. H. Carter for his valuable help throughout the whole series of experiments.

# A SIMPLE LABORATORY APPARATUS FOR THE CONTINUOUS EVAPORATION OF LARGE VOLUMES OF LIQUID *IN VACUO*.

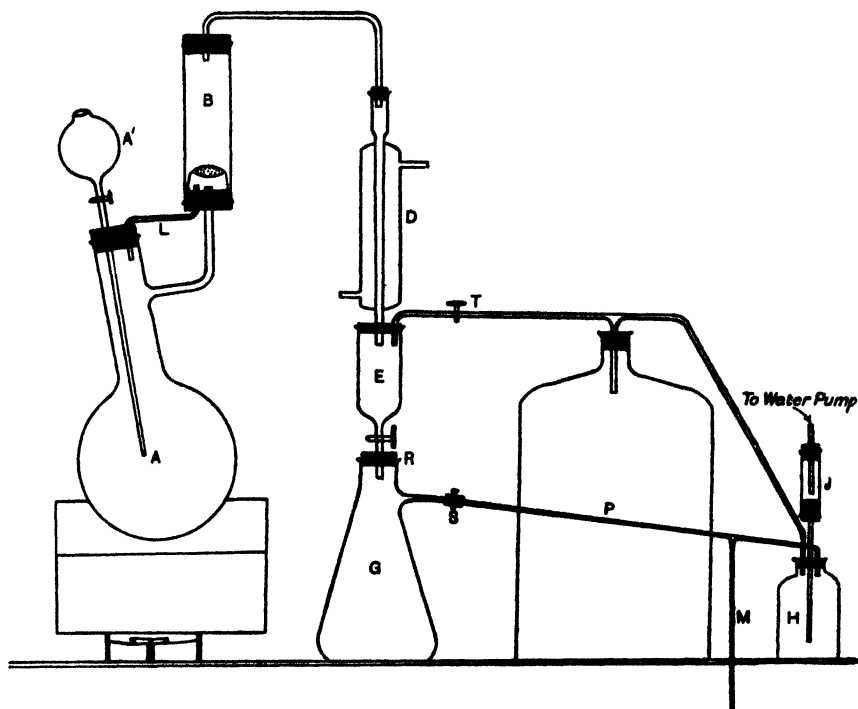
By WILLIAM A. DAVIS.

(*Rothamsted Experimental Station.*)

It is frequently necessary, especially in dealing with plant and animal extracts, to concentrate large volumes of liquid *in vacuo*. In such cases, the operation is often a very tedious one, owing to the necessity of closely watching the apparatus so as to control frothing and avoid the passing over of the liquid into the distillate. Having experienced this, more particularly in the distillation of alcoholic plant extracts, which show a great tendency to froth, the simple apparatus shown in the sketch has been devised which completely overcomes all the difficulties encountered in such work. By means of it large volumes of liquid can be evaporated continuously and the distillate recovered, if necessary in fractions; the apparatus requires practically no watching after the distillation has once been started, and the latter can be left to itself whilst other work is proceeded with. It is only necessary from time to time to renew the liquid in the distilling flask *A*, by means of the dropping funnel *A'*.

The apparatus consists of an ordinary distilling flask with the side-tube bent up and passing into a wide piece of glass tube *B* which serves as a froth-trap; the latter is connected by glass tubing with the condenser *D*, the lower end of which passes through a rubber stopper into the cylindrical dropping funnel *E*, which in turn is connected, as shown, below with the pump-flask *G*, and above with the large reservoir *P*, which serves to take up small variations of pressure and thus ensure a steady vacuum throughout the system.

In this way regular ebullition, without overheating or frothing, is secured.



The vacuum is maintained by means of an ordinary water injector-pump, connected through a Hutchinson regulating valve *J* (*Chemical News*, 1912, 99) with the bottle *H* and thence with *E* and *G*; a glass cock is interposed at *T*, whilst *S* is a screw-clamp which operates on the piece of rubber pressure-tubing connecting *G* and *H*. At *M* a manometer tube is inserted which shows the vacuum throughout the system. The Hutchinson valve takes up large variations in the vacuum due to changes of water pressure, so that by means of this, combined with the regulating reservoir *P*, changes in the vacuum are reduced to a minimum.

When the liquid in *A* first begins to boil there is often a great tendency to froth; should this occur, the froth rises into the trap *B*, breaks against a disc of copper-gauze, and the liquid is returned automatically to the flask through the piece of glass tube *L*.

The combination *E* and *G* allows of the distillate being removed from time to time; whilst the distillation is proceeding, the vacuum

in *G* is maintained the same as in the rest of the system, so that by opening the glass tap of *E* the distillate runs down into *G*. When *G* is full, and it is required to empty it, the cock on *E* is closed, and the screw-clamp *S* screwed down on to the rubber pressure-tube. The latter is then detached from the side tube of *G*, and the flask *G* removed from the rubber stopper *R*, emptied and replaced without interfering with the vacuum throughout the rest of the system. After it has been replaced, *S* is opened and in a very short time the vacuum is re-established in *G*, the same as throughout the rest of the apparatus.

It is a very simple matter by introducing T-pieces to run two or more of these distillation apparatus in conjunction with a single vacuum pump and a single regulating vessel *P*. We have had such an arrangement in continual use now for over a year and it answers all requirements.

All connections must of course be made with rubber stoppers or rubber pressure-tubing.

# A STUDY OF THE METHODS OF ESTIMATION OF CARBOHYDRATES, ESPECIALLY IN PLANT-EXTRACTS.

## A NEW METHOD FOR THE ESTIMATION OF MALTOSE IN PRESENCE OF OTHER SUGARS.

BY WILLIAM A. DAVIS AND ARTHUR JOHN DAISH.  
(*Rothamsted Experimental Station.*)

DURING an investigation of the carbohydrates present in the man-gold leaf, now in progress at the Rothamsted Experimental Station, we have made a special study of the methods of analysis applicable in such cases and have detected certain errors which are likely to occur in this class of work; although the investigation is still incomplete, it seems advisable to describe the methods which we have adopted to obviate these as far as possible.

The study of the accurate estimation of sugars in the complex mixtures occurring in plants may be said to date from the important memoir of Brown and Morris in 1893 entitled "A Contribution to the Chemistry and Physiology of Foliage Leaves"<sup>1</sup>; a few years later Brown, Morris and Millar published their tables<sup>2</sup> of the reducing power of pure dextrose, laevulose, maltose and invert sugar under certain defined conditions with varying concentrations of the sugars and determined the specific rotatory power of pure maltose. Quite recently Parkin<sup>3</sup> in studying the carbohydrates of the snowdrop leaf, which does not contain starch or maltose, tested certain points of analytical procedure necessary in dealing with plant-extracts.

### *Gravimetric Methods of Estimating Sugars.*

In the estimation of mixed sugars such as occur in plant extracts undoubtedly the most satisfactory gravimetric method is to work under

<sup>1</sup> *Trans. Roy. Soc.*, 1893, 63, 604.

<sup>2</sup> *Trans. Roy. Soc.*, 1897, 71, 72—123.

<sup>3</sup> *Biochem. J.*, 1912, 6, 1.



the conditions laid down by Brown, Morris and Millar, using the tables already referred to. We have tested the accuracy of these tables by means of carefully purified specimens of dextrose, laevulose, cane sugar and maltose, dried *in vacuo* at 105—106° (except the laevulose which was heated at 75—80° only) under the conditions adopted by Brown, Morris and Millar<sup>1</sup>, and have found a satisfactory agreement—that is to within 1 milligram on the weight of copper weighed, which Brown, Morris and Millar regard as the probable degree of accuracy of their method; when 0.20 to 0.35 grm. of copper is weighed the error is therefore within 0.5 per cent.

*Possible Error in the Gravimetric Method due to the Action of  
Fehling Solution on Asbestos.*

In our early experiments with purified sugars it was frequently found that the copper oxide weighed in duplicate experiments differed by very large amounts—often not by a mere milligram but by a centigram or more. It was at first thought that this was due to the use of a layer of asbestos in the Soxhlet or Gooch crucible which was insufficiently thick to retain the whole of the cuprous oxide during the filtration, although no oxide was visible in the filtered Fehling solution. On using a much thicker layer of asbestos ( $\frac{1}{2}$ " to 1"), however, as is usual in sugar-works

<sup>1</sup> Ost (*Chem. Zeit.*, 1897, **21**, 618) in reply to Brown, Morris and Millar's criticism of the value he had assigned to the specific rotatory power of maltose, based on a method in which the hydrated substance was weighed and the rotation for the anhydrous substance derived from this, threw doubt on their values, alleging that, when maltose is heated to a temperature above 95°, even *in vacuo* it begins to decompose, although without showing any external signs of change, the decomposition being indicated only by a falling off of rotatory power. According to Ost the values of the solution-densities and reducing powers given by Brown, Morris and Millar would therefore be only approximately correct ("können principiell nicht als exakt gelten") as slight change had probably occurred in the material used (dextrose, laevulose as well as maltose). This statement is reproduced in von Lippmann's *Chemie der Zuckerarten* (3rd edition, p. 1468), where preference is given to Ost's values of solution-densities.

We have not specially investigated this point but may point out that Ost makes use of a strange argument in support of his case, and refutes himself, when he maintains that the indication of decomposition having occurred in maltose is a *lowering* of the specific rotatory power and yet contends that Brown, Morris and Millar's *high* value for the specific rotation at 15.5° (137.98) as compared with his own (137.46) was due to this cause; had decomposition occurred a *lower* value would be expected. E. Schulze (*Chem. Zeit.*, 1902, **26**, 7) on the other hand maintains that maltose hydrate can be completely dehydrated at 100° in a current of air without any decomposition occurring, and Ling, Eynon and Lane (7th *International Congress App. Chem.*, 1910, **1**, 187) confirm Brown, Morris and Millar's tables of solution-densities. Our own results were also always in full accord with them.

(see Fröhling, *Anleitung zur Zuckerindustrie*, 7th edition, 1911, p. 112; von Lippmann in *Chem. Tech. Unterricht.-Methoden*, 5th edition, III, p. 403), it was found that the differences were thereby considerably increased. An example will show the character of the results obtained. Using successive portions of 25 c.c. of the same invert sugar solutions (prepared from pure cane sugar) otherwise treated in exactly the same way:

Soxhlet A (previously used for two charges) gave 0.3453 grm. CuO. Soxhlet B (also used for two charges) gave 0.3410 grm. CuO, but Soxhlet C (freshly packed with  $\frac{1}{4}$ " of asbestos and ignited) gave 0.3060 grm. CuO.

In this case the difference between the result C and results A and B is from 0.035 to 0.040 grm.

When thinner layers of asbestos were used in the Soxhlet tube smaller differences were observed, and it was found that by using approximately equal thicknesses of asbestos in different tubes results differing in duplicate by not more than a milligram could easily be obtained although these were far from being correct. An example may be given:

Taken 25 c.c. solution = 0.1356 grm. invert sugar.

*Thin asbestos layer.*

1. CuO = 0.3215 grm = 0.1343 invert sugar = 98.9 %.
2. CuO = 0.3211 ,, = 0.1341 ,, ,, = 98.7 %
3. CuO = 0.3188 ,, = 0.1330 ,, ,, = 98.0 %

*Thicker asbestos.* 25 c.c. of same solution.

1. CuO = 0.3090 grm = 0.1287 invert sugar = 94.8 %.
3. CuO = 0.3100 ,, = 0.1291 ,, ,, = 95.1 %

Herein lies probably the principal cause of the not infrequent disagreement between analysts dealing with sugar materials and the doubts which have been expressed as to the accuracy of gravimetric methods. The analyst, using approximately the same thickness of asbestos throughout his experiments, would obtain duplicates in close agreement although the actual result might be considerably at fault.

We became aware of this source of error by observing that, in the case of the particular variety of asbestos we were using (Kahlbaum's specially prepared long-fibre asbestos for Gooch crucibles), the loss of weight experienced with Fehling's solution was particularly pronounced. This asbestos, which when washed with 200 c.c. of boiling water containing 40 c.c. of concentrated nitric acid and subsequently with 300 c.c. of boiling water, showed practically no change in weight (not more than

0.0002 gm.), yet lost considerably on passing through it 50 c.c. of the hot Fehling solution as in an ordinary "Blank" made to determine the correction for self-reduction of the Fehling solution in Brown, Morris and Millar's method. Prior ignition of the asbestos did not alter this property. With thick layers of asbestos ( $\frac{1}{4}$ " to  $\frac{3}{4}$ " ) the loss of weight so caused amounted to several centigrams. When successive 50 c.c. quantities of Fehling solution were used, the loss of weight experienced with each successive charge rapidly diminished, and after about the third "Blank" there is the normal *gain* of 0.0015 to 0.0030 gm. CuO, corresponding with the correction necessary to introduce for self-reduction of the Fehling solution (Brown, Morris and Millar, *Trans.* 1897, 71, 96). It is clear that there is present in the asbestos, as an impurity, some easily decomposable silicate which is gradually dissolved away by the strongly alkaline Fehling solution. The following numbers illustrate this; they were obtained with layers  $\frac{1}{4}$ " to  $\frac{3}{4}$ " thick of the asbestos.

		Soxhlet 1	Soxhlet 2	Soxhlet 3
1st.	50 c.c. Fehling	- 0.0328	- 0.0800	- 0.0465
2nd.	" "	—	- 0.0020	- 0.0026
3rd.	" "	—	+ 0.0021	+ 0.0016

- indicates loss of weight, + gain of weight.

We find that by digesting the asbestos during 30 minutes with boiling 20 % sodium hydroxide, and then thoroughly washing with water, an asbestos is obtained which is quite suitable for use in a Gooch crucible or Soxhlet tube as it *undergoes no further perceptible loss when hot Fehling solution is passed through it*. Such asbestos gives the normal increase of 0.0015 to 0.0028 as the correction to be applied for the self-reduction of the Fehling solution, and on passing through it 100 c.c. of boiling 5 % sodium hydroxide the loss of weight is less than 0.0001 gm.

Different samples of asbestos differ widely in their behaviour with boiling Fehling solution or sodium hydroxide; we have not met with an asbestos which is completely unaffected by these solutions, sample C given below being the best we have as yet obtained. In most cases, the loss is very considerable. In the following examples the asbestos had previously been boiled with hydrochloric and nitric acids, and showed no loss under this treatment. The numbers show the percentage loss of weight on boiling during 30 minutes with 10 % sodium hydroxide.

A.	White, long fibre	loss 6.94 %
B.	White, long fibre	" 5.85 "
C.	White	" 0.18 "
D.	Blue	" 0.75 "

Although the error that may arise in this way is often very considerable, none of the standard works of analysis we have consulted refers to the necessity of a preliminary treatment of the asbestos with alkali, although von Lippmann (*Chem. Tech. Untersuch.-Methoden*, III. 403) quotes a reference to a paper by Casamajor (*Zeitsch. anal. Chem.*, **22**, 552) in which the ordinary treatment of asbestos with acids before use is recommended. In Lippmann's *Chemie der Zuckerarten* (3rd edition, p. 594) the necessity of using asbestos of "guter, reiner und langfaserigen Qualität" is stated and reference made to Maercker (*Oester. Ung. Zeit. für Zuckerind. und Landw.*, **7**, 699; *Zeitsch. Ver. Deutsch. Zuckerind.*, **28**, 797), Killing (*Zeit. angew. Chem.*, 1894, 431) and Elion (*Rec. Trav. Chem.*, 1896, **15**, 116), who had previously pointed out the necessity of using "pure" asbestos. Killing in 1894 went so far as to state that suitable asbestos bids fair to become very scarce or perhaps altogether to disappear from the market and recommended a return to the old method of collecting the cuprous oxide on filter paper.

*Weighing the Copper Precipitate from the Fehling Solution.*

Numerous papers have been published (cp. Lippmann, *Chemie der Zuckerarten*, 3rd Aufl. 596—598) in which the necessity of reducing the cuprous oxide to metallic copper and weighing as such have been emphasised; the simpler operation of oxidation has been frequently stated to give erroneous results owing to the difficulty of ensuring complete oxidation, the possibility of reduction owing to the action of the flame gases on the cupric oxide, and other causes<sup>1</sup>. Elion for instance (*Zeit. angew. Chem.*, 1890, 325) states that whereas by weighing as copper in sugar estimations he obtained close agreement in four experiments (0.2734, 0.2730, 0.2730, 0.2730), on weighing as cupric oxide he obtained widely discordant results (0.3328, 0.3068, 0.2962, 0.2854).

We have found, on the contrary, that the conversion of cuprous into cupric oxide is practically complete when the following precautions are observed.

1. The precipitate of cuprous oxide is collected in a porcelain Gooch crucible (with sufficient thickness of asbestos), finally washing

<sup>1</sup> In Moissan's *Traité de Chimie Minérale* (Vol. v. p. 459) the oxidation of cuprous oxide on heating in air is said to be incomplete on the authority of Grunhüt (*Chem. Zeit.*, 1894, **18**, 447), Nihoul (*Chem. Zeit.*, 1894, **18**, 881) and Killing (*Zeit. angew. Chem.*, 1894, 431). As we shall show, this is erroneous, unless the blow-pipe flame is used.

with alcohol and ether in the ordinary way, dried at 100°, and after placing in a No. 1 porcelain crucible to act as a container and prevent direct contact of the flame, heated in a fairly powerful flame from a  $\frac{1}{2}$ " Teclu burner or Fletcher Argand, until the weight is constant; generally, we simply keep the crucible over the flame during half an hour, allow to cool in the desiccator at least one hour, weigh and again heat another 30 minutes. The increase in weight in the second heating seldom exceeds 0.0005 grm.

2. *The blow-pipe should not be used*, as even when the Gooch is shielded by an outer crucible low results are obtained, probably owing to slight dissociation of the cupric oxide at the high temperature (compare Debray and Joannis, *Compt. Rend.*, 1884, 99, 383 and 688). Its use, too, is more tedious than that of a Teclu burner.

We append a few examples illustrating this. We have never observed the hygroscopic tendency which is sometimes attributed to cupric oxide.

	Wt. Cu <sub>2</sub> O	Wt. CuO	Ratio	Remarks
On blowpipe	1. 0.3730	0.4135	1.109	Heated on blow-pipe till constant in weight
	2. 0.3735	0.4140	1.109	
	3. 0.3864	0.4270	1.105	
	4. 0.2587	0.2857	1.105	
On Fletcher or Teclu burner	1. 0.3864	0.4297	1.112	
	2. 0.3767	0.4188	1.112	
	3. 0.3849	0.4271	1.110	
	4. 0.2410	0.2680	1.112	

$$\text{The theoretical ratio } \frac{2\text{CuO}}{\text{Cu}_2\text{O}} = 1.112 \text{ (Cu} = 63.57\text{)}.$$

A similar ratio  $\frac{\text{CuO}}{\text{Cu}_2\text{O}}$ , viz. 1.110 to 1.112, is always obtained when dealing with purified sugar solutions, but when the ordinary solutions obtained from plant extracts (previously treated with basic lead acetate and then deprived of the excess of lead) are used, the ratio is considerably lower (1.095—1.106); whilst it is lowest in estimating the reduction of solutions which have been treated with invertase or yeast, or starch solutions treated with malt extract (see below).

The recommendation is frequently made to weigh the cuprous oxide as such, after drying at 100°, and this method is prescribed for example in the Official and Provisional Methods of Analysis of the Association of Official Agric. Chemists (*U.S. Dept. Agric., Bull.* 107). (Compare Allen's

*Commercial Organic Analysis*, Vol. I. p. 325.) Whilst this course is safe in the case of solutions of pure sugars, it involves large error when dealing with solutions derived from plant or animal extracts, or when inversion or hydrolysis has been effected by an enzyme, or after fermentation by yeasts, even though alumina cream has been subsequently used to clear the solutions. In such cases the cuprous oxide invariably contains some organic matter, which burns away when it is oxidised, so that the ratio  $\frac{\text{CuO}}{\text{Cu}_2\text{O}}$  is thereby diminished<sup>1</sup>; it is probable that, in dealing with yeasts, invertase, etc., the cuprous oxide precipitated contains traces of copper salts of amino-acids as well as the colloidal organic matter carried down by adsorption. In such cases the cupric oxide weighed would be slightly higher than that actually due to reduction only; but the numerous experiments we have made with yeasts, invertase preparations, diastase, etc., would lead us to think that this error is relatively small, and negligible in comparison with other errors of sampling, etc., in this class of work.

We have made it a rule in our work to weigh both the cuprous and cupric oxide precipitate and calculate in each case the ratio  $\frac{\text{CuO}}{\text{Cu}_2\text{O}}$ . This throws an interesting light on the character of the solution dealt with, and on the purity of the cuprous oxide weighed. *But all calculations for sugars are based only on the weight of cupric oxide actually obtained.* We usually collect many successive charges in the same Gooch crucible one-after the other; the same Gooch can be used for 10—20 charges without cleaning, the cuprous oxide from a fresh experiment being collected on top of the previous charge of cupric oxide when the latter is constant in weight.

#### *Description of Heating Bath used.*

In order to facilitate the analyses we have made use of the form of water bath shown in the sketch; it consists of a 10" enamelled iron saucepan, 4½" deep, into which a false bottom of copper plate is placed, so as to afford a convenient support for the beaker flasks used. The cover of the bath is made of copper and consists of two halves, each

<sup>1</sup> Using invertase in the form of autolysed yeast the ratio of  $\frac{\text{CuO}}{\text{Cu}_2\text{O}}$  weighed varies between 1.060 to 1.090 according to the quantity used; with prepared diastase in starch transformations it is slightly higher (1.105—1.106) and with malt extract it again gives low figures similar to those obtained with autolysed yeast.

perforated with two  $2\frac{1}{4}$ " holes, the edge of the plate being turned down so as to fit over the bath. Each half of the cover can be lifted off separately so as to admit the beaker flask containing the Fehling solution. We find that 250 c.c. conical beaker flasks, with a top diameter  $2\frac{1}{4}$ " and bottom diameter  $2\frac{1}{8}$ ", give results in close agreement with the Brown, Morris and Millar tables, and are much more convenient for manipulation and heating than ordinary beakers.

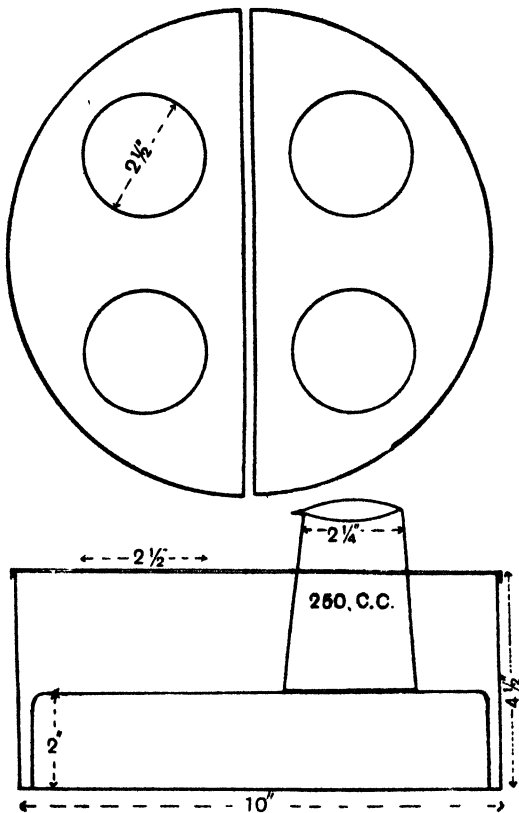


Fig. 1.

#### VOLUMETRIC METHODS.

We have spent some time in studying two of the volumetric methods which are generally regarded as the most accurate, viz. that due to Ling and Rendle (*Analyst*, 1905, **30**, 182; compare Ling and Jones, *Analyst*, 1908, **32**, 160), who make use of Fehling solution with ferrous thiocyanate as indicator; and the volumetric permanganate method as carried out by Bertrand (*Bull. Soc. Chim.*, 1906, [iii.], **35**, 1285).

Our sugar solutions were prepared with carefully purified dextrose (recrystallised several times from both methyl and ethyl alcohols)  $[\alpha]_D^{16} = 52.6^\circ$ , maltose  $[\alpha]_D^{15} = 138.0^\circ$  and invert sugar, prepared from cane sugar; in some cases the solutions were made by drying a known weight of the sugar *in vacuo* at  $106^\circ$  (toluene bath) in a small glass flask fitted with a ground-in stopper, and connected with a flask containing phosphoric anhydride, and then dissolving the anhydrous sugar in water and making to a known volume at  $15.0^\circ$ . In others, the concentration was checked by density determinations, using Brown, Morris and Millar's tables<sup>1</sup>.

*Ling-Rendle-Jones Method.*

- Dextrose.* 1. Taken 0.1975 grm. dextrose per 100 c.c. Found 0.1981 grm.  
2. Taken 0.2303 grm. dextrose per 100 c.c. Found 0.2300 grm.

*Cane Sugar.* (Inverted according to Ling and Rendle.)

Taken 0.2105 grm. per 100 c.c. Found 0.2106 grm.

*Maltose.* Taken 0.2059 grm. per 100 c.c. Found 0.2056 grm.

These confirm the view generally held that this method is accurate to at least 1 part in 300, or about 0.3 %, and we regard it as the most nearly accurate volumetric method at present in use. It is in our opinion preferable to the Bertrand method, both on the ground of accuracy and rapidity.

*Bertrand's Method.* This method, which consists in dissolving the cuprous oxide in an acid solution of ferric sulphate and titrating the resulting solution with permanganate, had in principle already formed the subject of at least six papers and was provisionally adopted by the U.S. Dept. of Agriculture at least as far back as 1899 (*Bulletin* 46, Bureau of Chemistry), prior to Bertrand's publication of convenient tables, which led to its being widely used in biochemical work.

We have carried out a large number of experiments with this method of which the following are typical: the solutions used were made from carefully purified sugars, and had given good results with the Brown, Morris and Millar gravimetric method and the Ling volumetric method. Bertrand's details were followed precisely.

*Dextrose.* Taken 0.0658 grm. dextrose.

Found in five experiments average 0.0648 grm., that is an error of

<sup>1</sup> In all cases our numbers refer to true c.c. at  $15^\circ$  C.



about 1.5 per cent. It is noteworthy that Bertrand gives his  $[\alpha]_D = 52.0^\circ$ , whereas the more nearly correct value is probably  $52.7^\circ$ .

*Cane sugar.* (Invert sugar.)

We obtained, using 0.0961 grm. invert sugar, results which were from 3 to 5 % low. The cause for this probably lies in the fact that the conditions for inverting cane sugar employed by Bertrand, namely heating 4.75 grms. of cane sugar with 50 c.c. of 2 % hydrochloric acid for 10–15 minutes at  $100^\circ$ , invariably leads to the decomposition of laevulose, which is shown by the production of a pronounced yellow colour in the solution; this is visible after 3 to 4 minutes heating. It is not safe to heat cane sugar with 2 % hydrochloric acid above  $70^\circ$ . In inverting cane sugar according to Bertrand's conditions, we invariably observed a decided yellow colour in our solution, whether simply boiled or heated in a boiling-water bath. That decomposition occurs at  $100^\circ$  has been generally recognised since the work of Herzfeld (*Zeit. Ver. Zuck.-Ind.*, 1898, 699 and 742).

*Maltose.* When working with 0.0824 grm. maltose we obtained results 0.0812 to 0.0826. Here the agreement is better, but the range of probable error is still large—nearly 1 %. It is noteworthy that Bertrand made use of hydrated maltose dried in a desiccator over sulphuric acid until constant in weight; to this the objection raised by Brown, Morris and Millar against Ost, that it contains slightly more than the theoretical 5 % of water of crystallisation, for  $1H_2O$ , can be applied. The  $[\alpha]_D$  given by Bertrand, viz.  $137.4^\circ$  (calculated for the anhydrous substance), as compared with what is probably the more correct value,  $138.0^\circ$ , agrees with this view.

In all the above experiments special care was taken to wash the cuprous oxide precipitate very thoroughly with 300–400 c.c. of boiling water, so as to remove the last traces of Fehling solution before adding the acid ferric sulphate solution. In all cases, too, the latter part of the operation was carried out as quickly as possible, so as to avoid the possibility of oxidation which, however, we satisfied ourselves by several experiments with acid solutions of ferrous sulphate is not to be feared under these conditions.

In our hands, the concordance between duplicate experiments made with this method was not such as is desirable in an accurate quantitative process. Bertrand speaks of this method as “un des plus pratiques et des plus précis,” an opinion which we cannot endorse; we regard it simply as a fairly rapid approximate method, which may perhaps in certain cases be useful when no high degree of accuracy is required.

In the case of cane sugar Bertrand's tables need revision. On the score of rapidity this method falls far short of the method advocated by Ling, Rendle and Jones.

#### METHODS OF INVERTING CANE SUGAR IN THE ESTIMATION OF SUGARS IN PLANT EXTRACTS.

In estimating cane sugar in plant extracts it is impossible to invert with hydrochloric acid at 70°, even under Herzfeld conditions as, if maltose is present, a considerable proportion also undergoes hydrolysis to dextrose (see p. 460) and there is also the danger of pentoses undergoing decomposition. It is therefore necessary to make use of invertase or a weak acid, such as citric acid or oxalic acid. We give the particulars of the invertase method later, and will first consider some of the difficulties which may arise in using citric acid.

Boiling 2% citric acid<sup>1</sup> has been frequently used for inverting cane sugar and was employed by Campbell (*J. Agric. Sci.*, 1912, 4, 248) in studying the carbohydrates of the mangold leaf. We can confirm the generally accepted view, that boiling during 10 minutes with 2% citric acid completely inverts cane sugar when alone.

1. Taken 1 grm. cane sugar, 4 grms. citric acid, 200 c.c. water, boiled 10 minutes, neutralised to phenolphthalein by sodium hydroxide and made up to 500 c.c.

Solution = 0.2105 grm. invert sugar per 100 c.c.

Found (Ling's method) 0.2089 grm. = 99.3% inversion.

2. A duplicate inversion.

Found (Ling's method) 0.2108 grm. invert sugar per 100 c.c. 100.1% inversion.

In a series of experiments carried out on mangold leaf extracts, from which tannins, bases, amino-acids, etc. had been removed by basic lead acetate in the usual way, it was found on estimating cane sugar by means of 2% citric acid that either cane sugar appeared to be entirely absent, or only a very small proportion seemed to be present. When, however, invertase was used with the same solution, the presence of a relatively large amount of this substance was disclosed. It was ultimately found that the cause of the difference in the two methods was due to the presence in the solution of a large proportion of sodium acetate, which almost entirely inhibits the inverting action of a 2% solution of citric acid.

<sup>1</sup> Throughout the percentage of citric acid we give refers to the percentage of the ordinary crystalline acid,  $C_6H_8O_7 + H_2O$ .

The sodium acetate was produced owing to the necessity of using a very large quantity of basic lead acetate in the removal of the amino-acids, etc., of the leaf; the slight excess of lead was precipitated by sodium carbonate, a relatively large proportion of which however was necessary to neutralise the acetic acid liberated by the amino-acids, tannins, etc. In this way the sugar solutions had become so enriched with sodium acetate as entirely to prevent inversion by citric acid of the concentration used (2%).

That this was actually the case is shown by the following experiments.

1. 20 c.c. of a cane sugar solution containing 0.7504 grm. cane sugar was mixed with 50 c.c. of water and 5 c.c. of the ordinary basic lead acetate solution (Allen's *Commercial Organic Analysis*, 4th edition, Vol. I. p. 308) and solid sodium carbonate gradually added so as to precipitate the lead but using as little sodium carbonate in excess as possible; the solution was then diluted to 100 c.c., and 25 c.c. of the filtrate (= 0.1876 cane sugar) neutralised to phenolphthalein by adding a few drops of a citric acid solution. 0.5 grm. of solid citric acid was then added, so as to make a 2% solution and the mixture boiled 10 minutes, after which it was cooled, neutralised with sodium hydroxide and heated with Fehling solution under Brown, Morris and Millar's conditions. *No weighable quantity of  $\text{Cu}_2\text{O}$  was obtained*, showing that under these conditions no inversion had occurred.

2. *Using Sodium Acetate only.*

It was calculated that 5 c.c. of the basic lead solution would give rise approximately to 1.13 grms. of sodium acetate,  $\text{C}_2\text{H}_3\text{O}_2\text{Na}$ ,  $3\text{H}_2\text{O}$ ; 40 c.c. of cane sugar solution (= 1.5008 grms.) and 2.26 grms. sodium acetate was diluted to 200 c.c. (Solution A), and 25 c.c. of this solution (= 0.1876 grm.) boiled during 10 minutes with 0.5 grm. citric acid. The solution was cooled, neutralised, and the reducing power estimated direct under Brown and Morris conditions:

$$\begin{aligned} 0.1389 \text{ CuO} &= 0.0562 \text{ invert sugar} = 0.0534 \text{ cane sugar} \\ &= 28.5\% \text{ inverted.} \end{aligned}$$

There had been some inversion, but nearly 75% of the cane sugar had been left intact.

Some experiments were made to ascertain the concentration of citric acid necessary to invert cane sugar in presence of considerable quantities of sodium acetate.

It was found that more than 80% of the cane sugar is inverted on boiling for 10 minutes with citric acid present to the extent of 2% if

normal sulphuric acid is first added until the appearance of the first indication of change of colour with methyl orange.

3. 25 c.c. of Solution A in 2 (= 0.1876 gm. cane sugar) + 1.6 c.c.  $N-H_2SO_4$  (first change of colour with methyl orange) + 0.532 gm. citric acid (= 2 %). Boiled 10 minutes, neutralised, and reducing power estimated.

$$\begin{aligned} \text{CuO} = 0.3973 \text{ gm.} &= 0.1701 \text{ invert sugar} = 0.1616 \text{ cane sugar} \\ &= 86.2 \% \text{ inverted.} \end{aligned}$$

4. Similar experiments were made with the solution obtained in 1 by adding basic lead acetate to the cane sugar solution and subsequently precipitating with sodium carbonate.

25 c.c. (= 0.1876 gm. cane sugar) treated with  $N-H_2SO_4$  (up to first indication of change of colour), solid citric acid added so as to give 2 % solution, boiled 10 minutes and neutralised.

$$(a) \text{ CuO} = 0.3713 \text{ gm.} = 0.1577 \text{ gm. invert sugar} = 0.1498 \text{ cane sugar} = 79.9 \% \text{ inverted.}$$

$$(b) \text{ CuO} = 0.3910 \text{ gm.} = 0.1670 \text{ gm. invert sugar} = 0.1586 \text{ cane sugar} = 84.6 \% \text{ inverted.}$$

The difference between the two experiments is probably due to a difference in the volume of sulphuric acid added, as the point of change with methyl orange is naturally very indistinct, owing to the sodium acetate present.

5. The same solution as in 4 was used. 25 c.c. (= 0.1876 gm. cane sugar) was neutralised to phenolphthalein by a concentrated citric acid solution, then an equal quantity of citric acid (to neutralise  $NaHCO_3$ ), and finally 1 gm. of solid citric acid added so as to invert with 4 % of the latter; boiled 10 minutes and neutralised.

$$(a) \text{ CuO} = 0.3142 = 0.1309 \text{ invert sugar} = 0.1243 \text{ cane sugar} = 66.3 \% \text{ inversion.}$$

$$(b) \text{ CuO} = 0.3288 = 0.1376 \text{ invert sugar} = 0.1302 \text{ cane sugar} = 69.7 \% \text{ inversion.}$$

Here the amount of inversion is less than in 4.

6. Adding  $N-H_2SO_4$  to first change with methyl orange and then citric acid to make exactly 4 %.

25 c.c. of solution 1 (deleaded by sodium carbonate) = 0.1876 gm. cane sugar + 2 c.c.  $N-H_2SO_4$  + 1.08 grms. solid citric acid. Boiled 10 minutes and neutralised with sodium hydroxide.

$$\begin{aligned} 0.4195 \text{ gm. CuO} &= 0.1811 \text{ gm. invert sugar} = 0.1720 \text{ cane sugar} \\ &= 91.76 \% \text{ inversion.} \end{aligned}$$

7. Same treatment as 6, but citric acid exactly 5% during inversion.

$\text{CuO} = 0.4292 = 0.1858$  gm. invert sugar = 0.1765 cane sugar = 94.1%.

Here inversion is still incomplete.

8. By adding sulphuric acid to first change, then citric acid to make a 10% solution and boiling 10 minutes, inversion is complete.

$\text{CuO} = 0.4500 = 0.1964$  invert sugar = 0.1865 cane sugar = 99.5%<sup>1</sup>.

*Inversion with invertase in presence of salts.*

It is remarkable that the action of invertase on cane sugar is not interfered with in the least by a proportion of sodium acetate which almost completely prevents inversion by 2% citric acid.

1. To 25 c.c. of the above solution of cane sugar (= 0.1876 cane sugar) containing 1.13% of sodium acetate, 1 c.c. of autolysed yeast was added and inverted for 2 hours at 40°. 3 c.c. of alumina cream was added, the solution filtered and the precipitate washed well, evaporating the washings so as finally to have 50 c.c. of the invert sugar solution for reduction under Brown, Morris and Millar conditions.

$\text{CuO} = 0.4515 = 0.1873$  gm. cane sugar inverted = 99.9%.

2. The same experiment was repeated, but making the cane sugar acid to litmus by adding one drop of *N*-sulphuric acid before adding the autolysed yeast.

$\text{CuO} = 0.4542$  gm. = 0.1885 gm. cane sugar = 100.6%.

A similar result was obtained by making the solution faintly acid to methyl orange with 1.6 c.c. of *N*-sulphuric acid before inversion; inversion was complete.

*Invertase and boiling citric acid do not hydrolyse maltose under the conditions used in dealing with plant extracts.*

It was important to make sure that in the inversion of cane sugar by either invertase or boiling citric acid, no maltose was hydrolysed to dextrose.

For this purpose carefully purified maltose was used (see p. 457), four times recrystallised from 80% alcohol; the solution was standardised by preparing a concentrated solution and ascertaining the density,

<sup>1</sup> No loss of sugar is therefore caused by the use of basic lead acetate, as has sometimes been stated to be the case. This supposed loss has been probably due to incomplete inversion, brought about by the presence of sodium acetate. Parkin has also shown that no loss occurs by a series of special experiments.

using Brown, Morris and Millar's tables, the value so obtained being confirmed by ascertaining the reducing power.

*Invertase.*

25 c.c. of maltose solution representing 1.0032 grms. anhydrous maltose was digested with 1 c.c. autolysed yeast for 3 hours at 40°; 5 c.c. of alumina cream was added, the solution filtered and washed to 100 c.c. with boiling water. Taken 20 c.c. = 0.2006 gm. maltose.

1.  $\text{CuO} = 0.2700 = 0.1979 \text{ gm. maltose} = 98.7\%$ .

2.  $\text{CuO} = 0.2716 = 0.1991 \text{ gm. maltose} = 99.3\%$ .

3.  $\text{CuO} = 0.2700 = 0.1979 \text{ gm. maltose} = 98.7\%$ .

The slight loss is probably due to retention of maltose by the alumina cream precipitate, in washing which only 70 c.c. of water was used. There has been no increase of reducing power such as would accompany hydrolysis of the maltose to dextrose.

*Action of boiling 10% citric acid on maltose.*

A solution of maltose containing 1.941 grms. maltose per 100 c.c. at 15° was used. 10 c.c. of this reduces 0.2642 gm. CuO.

1. 50 c.c. of the maltose solution (= 0.9705 gm. maltose) was boiled 10 minutes with 5 grms. solid citric acid, cooled, neutralised with sodium hydroxide to phenolphthalein and made up to 100 c.c.; taken 20 c.c. = 0.1941 maltose.

$\text{CuO} = 0.2768 = 0.1951 \text{ maltose} = 100.6\%$ .

Very slight hydrolysis of maltose had occurred, representing an increase of reduction of 0.0036 gm. CuO.

2. 50 c.c. of the same solution was made faintly acid to methyl orange, by adding a trace of sulphuric acid, then 5 grms. solid citric acid were added, and the solution boiled 10 minutes and treated as in 1.

Expt. 1.  $\text{CuO} = 0.2677$ .

Expt. 2.  $\text{CuO} = 0.2715$ .

Average = 0.2696 = 0.1976 maltose = 101.8%.

Slight hydrolysis of maltose occurred in these two separate experiments, representing an increase of 0.0035 gm. CuO in one case and 0.0073 in the other.

In another similar case a 1.0032 per cent. solution of maltose boiled with 10% of citric acid showed in two experiments an average value of 101.6% maltose. Slight hydrolysis had occurred.

3. Although under the conditions of 1 and 2 maltose showed a slight but distinct hydrolysis, it was found that, under the conditions actually existing in the analysis of plant products, when large quantities of basic lead acetate have to be used, and the excess of lead is removed by sodium carbonate, the sodium acetate formed is sufficient to inhibit all hydrolysis of maltose by boiling 10 % citric acid; the increase of reduction brought about by citric acid therefore represents true cane sugar. This is, of course, *not* the case if the lead is removed by hydrogen sulphide, because then the solution becomes strongly acid with acetic acid, and unless the acidity is neutralised (to phenolphthalein) by sodium hydroxide prior to the addition of citric acid, an even greater hydrolysis of maltose than is given above would be experienced.

*Maltose + sodium acetate and boiling 10 % citric acid.*

(i) 50 c.c. of maltose solution (= 0.5016 gm. maltose), boiled 10 minutes with 5.0 grms. solid citric acid + 0.565 gm. sodium acetate; cooled, neutralised with sodium hydroxide to phenolphthalein and made up to 100 c.c. at 15°.

(a) 25 c.c. taken :

(0.1254 maltose) = 0.1724 CuO = 0.1259 maltose = 100.4 % maltose taken.

(b) 40 c.c. taken :

(0.2006 maltose) = 0.2724 CuO = 0.1996 maltose =  $\frac{99.5\%}{99.95\%}$  maltose taken.

Here no perceptible hydrolysis has occurred.

(ii) The same is true if to the solution of maltose containing sodium acetate, sulphuric acid is added so as to make the solution just change colour with methyl orange.

75 c.c. maltose solution (= 0.7524 gm. maltose) + 0.847 gm. sodium acetate + 4.25 c.c.  $N \cdot H_2SO_4$  + 3.17 grms. citric acid. Boiled 10 minutes, neutralised and made up to 100 c.c.

25 c.c. = 0.1881 gm. maltose taken.

Found 0.2579 CuO = 0.1889 maltose = 100.4 %.

Practically no hydrolysis of maltose by 10 % citric acid is therefore to be feared. We have however invariably, in our analyses of plant materials, carried out the estimation of cane sugar both by invertase and 10 % citric acid. This as a general rule has given good agreement and a mutual check is thus obtained on the two methods: this procedure also ensures that the concentration of citric acid has been

sufficiently great to effect complete inversion. In certain cases, when very large quantities of basic lead solution have to be used, it might be necessary to employ citric acid of greater concentration than 10 %, but we have as yet not met with such necessity.

We have found that considerable hydrolysis of maltose occurs on treatment with hydrochloric acid under either the Clerget or Herzfeld conditions usual in the estimation of cane sugar; we have therefore not employed this acid at all in dealing with plant products. (Compare below, p. 458.)

### *Estimation of Maltose.*

It has been frequently proposed to estimate maltose by hydrolysis with dilute hydrochloric or sulphuric acid at 100°, noting the change of cupric reduction or specific rotatory power of the solution after allowing for the inversion of cane sugar present. Under carefully regulated conditions this method gives approximate results in the case of pure maltose or a mixture of maltose and dextrose (cf. Baker and Dick, *Analyst*, 1905, **30**, 79) but it is, as we shall show, inapplicable in all cases when cane sugar and laevulose or pentoses are present, as in the solutions prepared from plant extracts.

Brown and Morris in their classical paper of 1893 used hydrochloric acid under the conditions prescribed by Elion (*Zeit. angew. Chem.*, 1890, 291 and 321), 50 c.c. of the 1% solution being heated with 3 c.c. of concentrated hydrochloric acid for 3 hours at 100° (boiling-water bath). They observed that the "fall of angle on inversion with acid was, for some unexplained reason, always somewhat less than it ought to be on the supposition that it was due only to the hydrolysis of maltose," and suggested that this "probably indicates the presence of a small quantity of a hydrolysable substance other than maltose and with a less optical activity." In our early analyses of extracts of mangold leaves, we invariably observed the same phenomenon; but as the solutions always contained a brown humus-like precipitate, and had thus undergone considerable decomposition, it appeared that a probable explanation of the increased rotation lay in the destruction of a laevorotatory substance in the solution. The relative instability of laevulose in presence of acids suggested that this was the constituent undergoing change. Experiments with cane sugar and laevulose fully confirmed this view. (Tables I and II.)



TABLE I. *Action of 2.3 % HCl on Cane Sugar (2 hrs. heating at 100°).*

*Conditions.* 50 c.c. cane sugar solution + 25 c.c. water + 5 c.c. conc. HCl. After heating, neutralised and made to 100.4 c.c. Taken 20 c.c.

Sugar per 100 c.c. during heating	HCl/100 c.c. during heating	CuO weighed ex. 20 c.c.	Sugar found after heating per 100 c.c.	% sugar accounted for	Actual weight of sugar destroyed grms.
1.125	2.30	0.4066	0.8321	83.21	0.1679
"	"	0.4075	0.8350	83.50	0.1650

TABLE II. *Action of 2.44 % HCl on Laevulose (2 hrs. at 100°).*

*Conditions.* 10 c.c. laevulose solution (0.6560 laevulose) + 60 c.c. water + 5 c.c. HCl (36.6 HCl/100 c.c.); after heating 2 hrs. at 100° neutralised with sodium hydroxide and made to 100 c.c. at 15° C.

Conc. of laevulose during heating	HCl/100 c.c. during heating	CuO ex. 25 c.c.	Laevulose found after heating	Laevulose % destroyed	Actual laevulose destroyed
0.8840 grms. per 100 c.c.	2.44	0.2625	0.4520	31.1	0.2040 grms.
"	"	0.2614	0.4500	31.4	0.2060 "

In both cases, although the concentrations of acid and sugar are not strictly the same, there has been destruction of, roughly, the same quantity of laevulose; in the case of the pure laevulose the proportion of acid present was somewhat higher, thus accounting for the somewhat greater destruction of the sugar. A considerable quantity of dark brown humus-like substance separated from both solutions, which also became much discoloured, as had been experienced in dealing with the actual plant extracts.

It is clear, therefore, that laevulose is destroyed very largely under the conditions recommended by Brown and Morris for hydrolysing maltose. In the above experiments the heating was only carried out for 2 hours, whereas for the hydrolysis of maltose heating for 3 hours was recommended. We have not made an actual determination of the result of heating during 3 hours with the above concentration of acid, but Tables III and IV show the result of heating for 3 hours with a slightly higher concentration.

TABLE III. *Action of 4.71 % HCl on Cane Sugar at 100° (3 hours).*

50 c.c. of 2 % cane sugar solution (=1.000 grm.) + 15 c.c. water + 10 c.c. conc. hydrochloric acid. Heated 3 hrs. in boiling water, neutralised and made to 100 c.c. at 15° C. Used 20 c.c. for reduction.

Conc. of cane sugar during heating, per 100 c.c.	HCl/100 c.c. during heating	CuO from 20 c.c.	Cane sugar accounted for after heating*	% cane sugar destroyed	Actual laevulose destroyed grms.
1.333	4.71	0.2691	0.5310	46.9	0.4690
"	"	0.2691	0.5310	46.9	0.4690
"	"	0.2713	0.5355	46.45	0.4655
"	"	0.2710	0.5350	46.50	0.4650
		Average ...	0.5331	46.65	0.4669

\* Using dextrose reducing figure.

 TABLE IV. *Action of 4.58 % HCl on Laevulose at 100° (3 hours).*

10 c.c. of laevulose solution (0.6560 grm. laevulose) + 60 c.c. water + 10 c.c. conc. hydrochloric acid. Heated 3 hrs. in boiling water, neutralised and made to 100 c.c. Used 25 c.c. for reduction.

Conc. of laevulose during heating	HCl/100 c.c. during heating	CuO from 25 c.c.	Laevulose left	Laevulose % destroyed	Actual laevulose destroyed
0.8288	4.58	0.0339	0.0586	91.07	0.5974 grms.
0.8288	4.58	0.0518	0.0880	86.6	0.5680 "
		Made to 101.7 c.c.			

With both cane sugar and laevulose a considerable decomposition was made evident by the production of much brown, humus-like material. Laevulose is thus largely destroyed by heating with dilute hydrochloric acid for such prolonged periods as 2 to 3 hours at 100°. On the other hand, the figures for cane sugar would suggest that the dextrose remains mainly unchanged even after the more prolonged heating with nearly 5 % HCl. This point was specially tested, and Tables V and VI give the results.

TABLE V. *Action of 2.35 % HCl on Dextrose at 100°.*

20 c.c. dextrose solution (0.8216 grm.) + 50 c.c. water + 5 c.c. HCl conc. After heating neutralised and made to 100 c.c. at 15°.

Time of heating	Grms. dextrose per 100 c.c. during heating	Grms. HCl per 100 c.c. during heating	CuO ex. 20 c.c.	Dextrose found	Dextrose % found	Dextrose actually destroyed
2 hrs. at 100°	1.095	2.35	0.8948			
"	"	"	0.8955			
"	"	"	0.8950			
"	"	"	0.8948			
		Average...	0.8950	0.8140	99.0	0.0076 grms.

Dextrose in 1 % solution is only slightly decomposed by 2 hours' heating with 2.35 % HCl at 100°, although even here the dextrose actually destroyed is 7.6 mgrm., but when heated 4 hours in 4.38 % solution, with a greater concentration of acid (4.15 %) the amount of decomposition is considerable, the actual dextrose destroyed amounting to as much as 0.2400 grm. (Table VI).

TABLE VI. *Action of 4.15 % HCl on Dextrose at 100°.*

75 c.c. of dextrose solution (3.726 grms.) + 10 c.c. conc. HCl. Heated and made to 100 c.c. 20 c.c. diluted to 100 c.c. and 25 c.c. taken for reduction.

Time of heating	Grms. dextrose per 100 c.c. during heating	Grms. HCl per 100 c.c. during heating	CuO ex. 25 c.c.	Dextrose found	Dextrose % found	Dextrose actually destroyed
4 hrs. at 100°	4.38	4.15	0.4180			
"	"	"	0.4195			
		Average...	0.4188	3.486	98.6	0.2400

It was hoped that it would be possible to arrange the conditions for the hydrolysis of maltose by dilute acid so as at the same time to leave the laevulose intact, but the following experiments showed that it was only possible to obtain anything like complete hydrolysis of maltose under conditions which bring about considerable destruction of laevulose.

TABLE VII. *Hydrolysis of Maltose\* by Hydrochloric Acid at 100°.*

20 c.c. maltose solution (=0.8226 grm.) + 5 c.c. conc. HCl + 50 c.c. water. After heating, neutralised and made to 100 c.c.

Time of heating	Grms. maltose per 100 c.c. during heating	Grms. HCl per 100 c.c. during heating	CuO ex. 20 c.c.	Maltose found per 100 c.c.	Maltose % calculated from dextrose formed
1 hour	1.097	2.35	0.4060		
"	"	"	0.4057		
		Average...	0.4058	0.7983	97.05†
2 hours	1.097	2.35	0.4096		
"	"	"	0.4077		
		Average...	0.4087	0.8051	97.88
3 hours	1.097	2.35	0.4080		
"	"	"	0.4091		
		Average..	0.4086	0.8045	97.82

\* For the purpose of our experiments Kahlbaum's maltose was recrystallised several times from 80 % alcohol; the commercial material contains generally from 10 to 15 % of dextrin and great care is required in recrystallisation to remove this. We dissolve the maltose in 80 % alcohol, and leave to cool, when the dextrin separates as an oily layer from which the solution of the purer sugar is decanted and the process repeated. Our finally purified material had almost identically the same physical properties and the same reducing power as given by Brown, Morris and Millar (*Trans.*, 1897), within the error of 0.5 %.

25 c.c. of a solution containing 0.8226 grm. anhydrous maltose (dried *in vacuo* at 105°) per 100 c.c. gave

$$(1) \quad 0.2809 \text{ CuO} = 0.2059 \text{ maltose} = 100.1 \%$$

$$(2) \quad 0.2821 \text{ CuO} = 0.2067 \text{ " } = 100.5 \%$$

† Baker and Dick after 90 minutes' heating found with 1 % maltose + 20 c.c. water + 1 c.c. conc. HCl (sp. gr. 1.16) a hydrolysis of 96.5 %.

The maltose was heated with hydrochloric acid for 1, 2 and 3 hours in a long-necked flask fitted with a reflux and heated in boiling water; before hydrolysis the maltose was approximately 1 % and the acid 2.35 %.

Hydrolysis is not complete after 1 hour's heating at 100°, as is shown by the slight increase in reduction after 2 hours' heating; the increase in hydrolysis is however balanced by the increasing destruction of dextrose, which after 2 hours, according to p. 456, becomes distinctly noticeable.

With slightly stronger acid and longer periods of heating, *lower* results are obtained for maltose, as might be anticipated from the destruction of dextrose which occurs (compare Table VI, p. 456); the following results illustrate this:

TABLE VIII. *Hydrolysis of Maltose by 4.15 % HCl at 100°.*

75 c.c. dilute maltose solution (=0.6171 grm.) + 10 c.c. conc. HCl; after heating at 100° neutralised and made to 100 c.c.

Time of heating	Grms. maltose per 100 c.c. during heating	Grms. HCl per 100 c.c. during heating	CuO ex. 20 c.c.	Maltose found per 100 c.c.	Maltose % calculated from dextrose formed
3 hours	0.7261	4.15	0.3105		
"	"	"	0.3117		
"	"	"	0.3119		
		Average...	0.3114	0.5905	95.70
4 hours	0.7261	4.15	0.3068		
"	"	"	0.3064		
"	"	"	0.3061		
		Average...	0.3064	0.5805	94.06

*Hydrolysis of Maltose at 70°.*

Hydrolysis of maltose in 1 % solution by 2.44 % hydrochloric acid at 70°, is very slow, and even after 24 hours' heating only 94 % is converted into dextrose; it is possible that slight decomposition of the dextrose formed may take place during this prolonged period, but this point was not specially pursued, as it was found that laevulose certainly underwent considerable decomposition under these conditions and to an extent rendering it impossible accurately to estimate maltose in presence of cane sugar and laevulose by hydrolysis at this temperature.

The solutions remained colourless throughout the whole time of heating. In calculating the percentage of maltose converted in the above hydrolyses it must be remembered that the reducing power represented by CuO is due to a mixture of dextrose and unconverted maltose. In the last three experiments the original concentration of maltose is slightly different from that of the first three experiments, but the concentration of hydrochloric acid is maintained the same.

TABLE IX. *Action of 2.44 % Hydrochloric Acid on Maltose at 70°*

20 c.c. of maltose solution (0.7620 gm.) + 50 c.c. water + 5 c.c. conc. HCl ;  
after heating neutralised and made to 100°.

Time of heating	Grms. maltose per 100 c.c. during heating	Grms. HCl per 100 c.c. during heating	CuO ex. 25 c.c.	% maltose converted
2 hrs. at 70°	1.016	2.44	0.3198	28.85
"	"	"	0.3193	
		Average...	0.3195	
3 hrs. at 70°	1.016	2.44	0.3320	34.3
"	"	"	0.3328	
		Average...	0.3324	
6 hrs. at 70°	1.016	2.44	0.3705	52.4
"	"	"	0.3693	
		Average...	0.3700	

20.69 of another maltose solution (0.7186 gm.) + 49.3 c.c. water  
+ 5 c.c. HCl conc., as above.

12 hrs. at 70°	1.042	2.44	0.4449	83.3
"	"	"	0.4447	
		Average...	0.4448	
18 hrs. at 70°	1.042	2.44	0.4635	92.1
"	"	"	0.4635	
		Average...	0.4635	
24 hrs. at 70°	1.042	2.44	0.4673	93.9
"	"	"	0.4672	
		Average...	0.4673	

TABLE X. *Action of 4.58% Hydrochloric Acid on Maltose at 70°.*

20 c.c. of maltose solution (=0.9150 grm. anhydrous maltose) + 50 c.c. water + 10 c.c. conc. HCl was heated 3 and 6 hours at 70°. The solution was neutralised with sodium hydroxide and made to 100 c.c. at 15°. The reducing power was estimated in 20 c.c.

Time of heating	Conc. of maltose during heating grms. per 100 c.c.	HCl per 100 c.c. during heating	CuO ex. 20 c.c.	% Maltose converted
3 hours	1.144	4.58	0.3955	69.9
"	"	"	0.3916	
		Average...	0.3935	
6 hours	1.144	4.58	0.4300	87.6
"	"	"	0.4300	
		Average...	0.4300	

The curves summarise the above results.

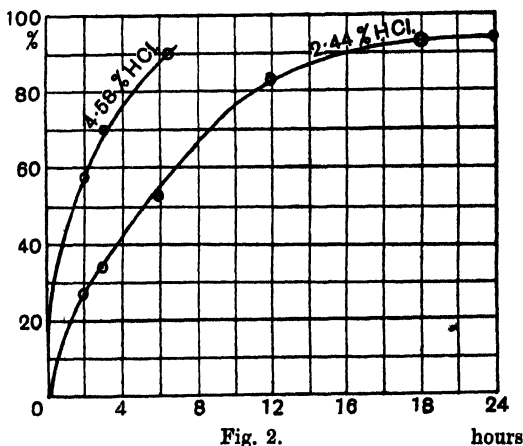


Fig. 2.

*Action of Hydrochloric Acid on Dextrose at 70°.*

The following results show that dextrose is only very slightly changed by prolonged heating (36 hours) with 2.44 hydrochloric acid at 70°; but stronger acid (4.88%) brings about noticeable decomposition in 24 hours.

TABLE XI. *Action of 2.44 % HCl on Dextrose at 70°.*

20 c.c. dextrose solution (0.7640 gm. dextrose) + 50 c.c. water + 5 c.c. HCl (36.6 HCl/100 c.c.). Heated 36 hrs. at 70° C.; neutralised with sodium hydroxide and made to 100 c.c. at 15° C. Conc. of dextrose during heating = 1.0187 gm. per 100 c.c.

HCl/100 c.c. during heating	CuO ex. 25 c.c.	Dextrose found after heating	Dextrose % accounted for	Actual dextrose destroyed
2.44	0.4380	0.7608	99.6	0.0032 grms.

TABLE XII. *Action of 4.88 % HCl on Dextrose at 70°.*

20 c.c. dextrose solution (0.7640 gm. dextrose) + 45 c.c. water + 10 c.c. HCl (36.6 HCl/100 c.c.). Heated 24 hrs. at 70° C.; neutralised with sodium hydroxide and made to 100 c.c. at 15° C. Conc. of dextrose during heating = 1.0187 gm. per 100 c.c.

HCl/100 c.c. during heating	CuO ex. 25 c.c.	Dextrose found after heating	Dextrose % accounted for	Actual dextrose destroyed
4.88	0.4341	0.7532	98.6	0.0108 grms.

TABLE XIII. *Action of 2.44 % Hydrochloric Acid on Laevulose at 70°.*

10 c.c. laevulose solution (0.6560 gm. laevulose) + 60 c.c. water + 5 c.c. conc. HCl. Heated under reflux for 2, 18 and 24 hours at 70°. After heating, neutralised with sodium hydroxide and made to 100 c.c. at 15°. Used 25 c.c. for reduction.

Time of heating	Grms. laevulose per 100 c.c. during heating	Grms. HCl/100 c.c. during heating	CuO ex. 25 c.c.	Laevulose found	Laevulose % left	Laevulose destroyed grms.
2 hours	0.8840	2.44	0.3687	0.6524	99.50	0.0036
"	"	"	0.3696	0.6540	99.73	0.0020
		Average...	0.3691	0.6532	99.62	0.0028
18 hours	0.8840	2.44	0.3605	0.6368	97.1	0.0192
"	"	"	0.3602	0.6360	97.0	0.0200
		Average...	0.3603	0.6364	97.05	0.0196
24 hours	0.8840	2.44	0.3530	0.6224	94.9	0.0336
"	"	"	0.3504	0.6160	93.9	0.0400
		Average...	0.3517	0.6192	94.5	0.0368

In 0.8840 % solution laevulose is only very slightly destroyed by 2 hours' heating with 2.44 % hydrochloric acid at 70°; but longer



periods of heating bring about considerable destruction, so that it is impossible to hydrolyse maltose even at 70° in presence of laevulose, without destruction of the latter.

TABLE XIV. *Action of 4.88 % Hydrochloric Acid on Laevulose at 70°.*

10 c.c. laevulose solution (0.6560 grm.) + 55 c.c. water + 10 c.c. conc. HCl. Heated 6 hours at 70°. Neutralised and made to 100 c.c. at 15°; taken 25 c.c. for reduction.

Time of heating	Grms. laevulose per 100 c.c. during heating	Grms. HCl per 100 c.c.	CuO ex. 25 c.c.	Laevulose found	Laevulose % left	Actual laevulose destroyed
6 hours	0.8840	4.88	0.3520	0.6200	94.5	0.0360
"	"	"	0.3552	0.6272	95.6	0.0288
		Average...	0.3536	0.6236	95.1	0.0324

In this case the destruction of laevulose in 6 hours is almost as great as with the 2.44 % acid in 24 hours.

*Estimation of Maltose in presence of other Sugars by means of Maltase-free yeasts.*

It has been known for some years that certain species of yeast do not contain maltase and hence are incapable of fermenting maltose; owing to the impossibility of estimating this substance in presence of the other sugars likely to be present in plant extracts by means of the ordinary methods we decided to ascertain whether these yeasts are suitable for purposes of quantitative estimation of this sugar<sup>1</sup>.

For this purpose we fermented a solution of maltose both alone and mixed with cane sugar, with pure cultures of *S. exiguus*, *S. anomalus* and *S. marxianus*, which Dr H. B. Hutchinson was good enough to prepare for us. To the solution of sugar, 5 c.c. of yeast water was added and the mixture, after being sterilised by 10 minutes' heating in the autoclave, was inoculated with a trace of the pure yeast from an agar-yeast-water tube-culture.

<sup>1</sup> Baker and Dick (*Analyst*, 1905, 80, 79) have suggested the use of *S. marxianus* for detecting maltose in presence of dextrose, by the increase of specific rotation and drop in reducing power which occur in fermenting the mixed sugars with this yeast; they fermented, however, only for a relatively short time and did not completely remove the dextrose as we have done, so as to make the method a quantitative one.

The flask containing the liquid is stoppered with cotton wool in the usual manner and incubated at 25° for three to four weeks. When the fermentation is complete, 5 c.c. of alumina cream is added and the solution boiled to expel alcohol: it is then filtered, and the precipitate washed until the filtrate has a volume of 100 c.c.

An aliquot portion (50 c.c.) can then be used for the cupric reduction.

TABLE XV.

	Yeast	Time	CuO from 50 c.c.	Maltose found	% Maltose found
<b>1st Series</b>					
<b>Maltose (0·2006) + Cane Sugar (0·3751)</b>	<i>S. exiguus</i>	17 days	0·1525	0·2224	110·8
	<i>S. anomalus</i>	23 "	0·1355	0·1974	98·4
	<i>S. marxianus</i>	21 "	0·1348	0·1962	97·4
<b>Maltose only</b>	<i>S. anomalus</i>	21 "	0·1360	0·1980	98·7
	<i>S. marxianus</i>	21 "	0·1340	0·1952	97·3
<b>2nd Series</b>					
<b>Maltose (0·3704) + Cane Sugar (0·2000)</b>	<i>S. exiguus</i>	31 "	0·2535	0·3712	100·2
	<i>S. marxianus</i>	31 "	0·2548	0·3736	100·8
	<i>S. marxianus</i>	31 "	0·2547	0·3734	100·7

In the first series the high result with *S. exiguus* is undoubtedly due to incomplete fermentation, the time being insufficient. The slightly low results in the other experiments in this series are due either to the maltose used being slightly contaminated with dextrose, or, more probably, to experimental error on the relatively small quantity of maltose taken. In the second series a very carefully purified maltose was used and a larger quantity taken so as to minimise the proportional error. In this case it will be seen that in spite of a very vigorous growth of yeast the maltose is quantitatively recovered, whilst the cane sugar is completely fermented away.

By the use of these special maltase-free yeasts it is therefore possible accurately to estimate maltose in presence of other sugars (dextrose, laevulose and cane sugar) which are completely fermented by them. We have applied this method now for some considerable time to the analysis of plant extracts and find that it is generally necessary also to introduce a correction for the presence of reducing

substances such as pentoses<sup>1</sup> which remain after fermentation by the yeast is complete. This correction is obtained by carrying out fermentation with a pure culture of ordinary distillery or baker's yeast; which ferments away the maltose as well as the other sugars, but leaves a slight residual reduction due to pentoses, etc.; on subtracting this value from the reducing value obtained by using the maltase-free yeasts, the cupric reduction due to maltose alone is obtained.

### *Estimation of Maltose in Plant-extracts.*

For this purpose the plant extract, from which amino-acids, tannins, etc., have been removed by means of basic lead acetate, has to be entirely freed from lead before the yeasts will grow satisfactorily. For this purpose two methods may be used:

1. Solid sodium carbonate is added little by little until no further precipitate is produced. The filtrate, which still contains traces of lead, is made slightly acid with hydrochloric acid and treated with hydrogen sulphide as in 2.

2. The excess of lead is removed directly by hydrogen sulphide. In this case the solution becomes strongly acid owing to the presence of free acetic acid and must be partly neutralised by adding dilute sodium carbonate solution until the reaction is faintly but distinctly acid to litmus paper (see p. 467).

It has been our custom to carry out five fermentations with each plant extract to be analysed, viz. one each with *S. anomalus*, *S. exiguus* and *S. marxianus*, and two with distillery yeast. The agreement between the results with the different special yeasts has generally been entirely satisfactory. Certain differences however in the behaviour of the yeasts may here be noted.

*S. anomalus* grows rapidly and gives a very bulky mass of yeast; it is apparently less efficient as a sugar remover than *S. marxianus* or *S. exiguus*, that is, it is slower in its action, and a greater yeast growth accompanies the removal of a certain weight of sugar. It shows a decided tendency to grow at the surface as a film. The cuprous oxide obtained in the subsequent reductions often filters very slowly.

*S. marxianus* is more sensitive to slight excess of acid than the

<sup>1</sup> The pentoses present in plant extracts are apparently not fermented by either baker's yeast or the special maltase-free yeasts we have used. Experiments on this point are still in progress.

other two yeasts, and refuses to grow in acid solutions in which the others readily multiply. *S. exiguus* is probably the most convenient for general use.

### *Pentoses.*

Pentoses are generally present to some considerable extent in the solutions obtained by the extraction of foliage leaves, and, after the treatment with basic lead acetate and subsequent removal of excess of lead, exercise a reducing action on Fehling solution. In view of recent work, especially that of Levene and Jacobs (*Ber.*, 1909, **42**, 2469, 2474, 2703; *Biochem. Zeit.*, 1910, **28**, 127), it is probable that pentoses play an extremely important part in the leaf's activity, especially *d*-ribose, which is an essential constituent of the nucleus of both plant and animal cells; the pentoses present in the aqueous alcoholic extract of leaf tissue are very possibly largely derived from nucleic acids. There is, however, also the possibility of the presence of arabinose and xylose as well as methylpentoses. In calculating the proportions of dextrose and laevulose we therefore have to make allowance, after subtracting the reduction due to the maltose (when this is actually present), for the pentoses; here we are faced with the difficulty that we do not know in any particular case what pentoses actually are present or what is their reducing power under the special conditions of the actual analysis.

In the present state of our knowledge we must be content with approximations, but the nature of these will affect the accuracy of the values for dextrose and laevulose, which are calculated from the primary reducing power of the plant solutions.

We have in our experiments estimated the total pentoses by distilling a quantity of the solution of which the "direct" reducing power of the sugars is determined, with hydrochloric acid according to the ordinary A.O.A.C. method (see Allen's *Commercial Organic Analysis*, I. 401; Bulletin 107, U.S.A. Dept. of Agriculture), weighing the furfural formed as phloroglucide. We intend to introduce a correction for the pentoses by ascertaining their reducing power under the conditions in which we have made our analyses.

*Analysis of Plant Extracts.*

The scheme of the analysis of a plant extract, such as that of foliage leaves, which we have adopted, may be outlined as follows:

Extract evaporated *in vacuo* (700 to 740 mm.) to small volume. Made to a definite volume, say 500 c.c.

2 portions of 20 c.c. each evaporated to dryness and dried <i>in vacuo</i> , 18 hours at 100°. This gives total dry matter in extract. (20 c.c. kept as reserve in case of accident.)	440 c.c. treated with basic lead acetate, filtered under pressure on Büchner funnel and washed to known volume (2 litres) = Solution A.
---	---

300 c.c. of Solution A is deleded by solid  $\text{Na}_2\text{CO}_3$  and made up to 500 c.c. = Solution B.

1. 25 c.c. of B is used for *direct reduction* and *polarised*<sup>1</sup>; the reduction is due to *dextrose*, *laevulose*, *maltose*, *pentoses*.

2. For cane sugar. 50 c.c. of B is inverted:

(a) By *invertase*. Make faintly acid to *methyl orange* by a few drops of concentrated sulphuric acid, and add 1—2 c.c. autolysed yeast and two or three drops of toluene and leave 24 hours at 38—40°C. After this period, add 5 to 10 c.c. alumina cream, filter and wash to 100 c.c. Take the reducing power of 50 c.c. (= 25 c.c. B) and polarise.

(b) By 10% *citric acid*. Make faintly acid to *methyl orange* by a few drops of conc. sulphuric acid and add a weighed quantity of citric acid crystals so as to have 10% of the crystalline acid ( $\text{C}_6\text{H}_8\text{O}_7 + \text{H}_2\text{O}$ ) present. Boil 10 minutes, cool, neutralise (to phenolphthalein) with sodium hydroxide, make to 100 c.c. and determine *reducing power* of 50 c.c. (= 25 c.c. B). Polarise.

*Cane sugar* is calculated from the increase of reducing power or change of rotation caused by inversion. The values obtained by the two methods *a* and *b* should agree closely.

3. For *maltose*. Another 300 c.c. of Solution A is deleded by means of hydrogen sulphide and filtered, the precipitated sulphide being washed until the total volume of filtrate and washings is about

<sup>1</sup> The polarisation of these dilute solutions is usually small and it is therefore necessary to take the reading with a long tube (at least 200 mm. in length), with an instrument reading accurately to  $\frac{1}{100}^\circ$ , the temperature being maintained constant at 20° C. within  $\frac{1}{10}^\circ$ . It is an easy matter, using a Lowry thermo-regulator, and circulating the water by means of a small pump, to keep the temperature constant to  $\frac{1}{100}^\circ$ ; but we find that differences of temperature less than  $\frac{1}{10}^\circ$  hardly make a perceptible difference in the readings with such dilute solutions as we have worked with.

450 c.c. Air is then sucked through this for about  $1\frac{1}{2}$  hours to expel hydrogen sulphide, a very little ferric hydroxide is added to remove the last traces of the latter, and the solution is made to 500 c.c. It is filtered and

50 c.c. fermented	(a)	with	<i>S. marxianus</i>
"	"	(b)	" <i>S. anomalus</i>
"	"	(c)	" <i>S. exiguus</i>

and two lots *d* and *e* of 50 c.c. are fermented with baker's yeast. It is generally necessary, in order to ensure good growth of the yeast, to reduce the acidity by adding 2 to 5 c.c. of *N*-sodium carbonate to the 50 c.c. to be fermented; 5 c.c. of sterilised yeast water is also added, the mixture is sterilised in the usual way and inoculated in the inoculating chamber with the pure culture of yeast. It is then stoppered with cotton wool and the yeast allowed to incubate for 21 to 28 days at 25°.

After completion of fermentation, 5 c.c. alumina cream is added, the solution made to 100 c.c. at 15°, filtered and 50 c.c. used for reduction. The *difference* between the *average* reduction with *a*, *b*, *c* and the average of *d* and *e* gives the reduction due to *maltose*.

4. *Pentoses*. These are approximately determined in 50 c.c. of A by distilling with hydrochloric acid according to the A.O.A.C. method, weighing as phloroglucide.

5. When the reduction in 1 due to pentose and maltose has been allowed for, the remaining direct reduction is due to dextrose and laevulose; the actual proportions of these two sugars are calculated from the reducing power combined with the corrected specific rotation as suggested by Brown and Morris in their 1893 paper.

In conclusion, we wish to express our best thanks to Dr H. B. Hutchinson for his kindness in preparing pure cultures of the yeasts employed and assistance in their manipulation and to Mr G. C. Sawyer for help in the analyses.

### *Summary.*

1. Certain sources of error encountered in the estimation of sugars in plant extracts are dealt with. Large errors in the gravimetric method may be obtained unless special care is taken in purifying the asbestos by boiling for at least 30 minutes with 20 % sodium hydroxide. Weighing the reduced copper as cuprous oxide is likely to give rise to

large error, and a process of weighing as cupric oxide, with certain precautions, is recommended.

2. The volumetric methods of Ling and of Bertrand have been studied; the former is preferable in all respects to the latter, which we regard as only roughly approximate.

3. In dealing with plant extracts, owing to the accumulation of sodium acetate in the solutions analysed, inversion by citric acid of lower concentration than 10 % is generally incomplete. Inversion by invertase is, however, not interfered with by this salt. To estimate cane sugar inversion both by invertase and 10 % citric acid is recommended. No loss of sugars occurs owing to the use of basic lead acetate as has been sometimes stated; the supposed loss is probably due to incomplete inversion caused by the presence of sodium acetate.

4. It is shown by a detailed study of the action of dilute hydrochloric acid on different sugars that it is impossible completely to hydrolyse maltose at either 70° or 100° without simultaneously destroying large quantities of laevulose or dextrose.

5. The only available method for the accurate estimation of maltose consists in the employment of special maltase-free yeasts, such as *S. exiguus*, *S. marxianus* or *S. anomalus*, introducing a correction (for pentoses, etc.) obtained by a special fermentation with baker's or brewer's yeast.

6. A scheme for the quantitative estimation of sugars in plant material is given.

# THE MOVEMENTS OF SOIL-WATER IN AN EGYPTIAN COTTON-FIELD.

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THE attention of all scientists in Egypt has been more and more closely directed to the problems of Soil-water since about the year 1907. In the first instance this attention was of necessity devoted to the water-logged layer of the soil and sub-soil, to the fluctuations of this layer in time and in space, and to its effects on the cotton plant.

The main outlines of this problem having been clearly distinguished, and due recognition made of the important fact that the growth of cotton is more usually "limited" by the soil-water than by any other factor, except during the first half of development, it was natural that attention should then be turned to the effects of variations in the water-content of soils above the saturated layer, and to the physical problems involved, with the object of obtaining knowledge which could be translated into irrigation practice.

Work on these latter lines has been commenced by other workers, but since the problem lies, in part at least, within the domain of the plant physiologist, the writer has attacked it independently with the object of tracing a base-line from which less minute observations might be worked out. The results completely confirm the general statement of the case from the botanical view-point which the writer has made elsewhere<sup>1</sup>, and also provide some data in respect of soil-physics.

## EXPERIMENTAL METHODS.

*Site and area.* A random choice was made of an area ten metres by twenty in a field surrounding the Botanical Laboratory at Giza. This area was surrounded by similar land in all directions. The land

<sup>1</sup> Balls, W. Lawrence, *The Cotton Plant in Egypt*, London, 1912.



was sown in Assili cotton, planted at the usual spacing of the district, namely, 45 cm. apart on ridges at 75 cm., with two plants left in each hole. Cultivation was conventional, and an average yield was obtained from the land, to wit, about 500 pounds of lint per acre.

The small size of the area actually sampled was almost inevitable, on account of the great diversity of sub-soil which may be encountered in adjacent borings. This applies to any alluvial soil, and particularly to the land around our laboratory, where the junction between a bed of loam and a bed of clay has been found to slope half a metre in ten metres. By repeated sampling of the same area on a systematic scatter, it was possible to eliminate these diversities almost completely.

A plan was kept which showed the exact position of each bore.

Within this area of 200 sq. m. the geological structure of the soil was almost constant, in the following way :

Surface to 30 cm. <sup>1</sup>	...	...	Made soil.
30 cm. to 90 cm.	...	...	Stiff clayey soil.
90 cm. to 200 cm.	...	...	Loam to sandy loam.
Below 200 cm.	...	...	Stiff clayey soil.

The clayey soils are not true clays, but are of similar physical properties, owing to the fineness of their particles. When their water-content is reduced to 10 % they are almost impenetrable with a Fraenckel borer, and the only interruption of the series here described was due to a breakage of the boring tool from this cause.

*Sampling.* The samples were taken at every twenty centimetres, from 20 cm. to 160 cm., one bore of eight samples being taken on each date. Two bores were made each week from May 4th to September 28th, or five months in all.

The mean dry-weight of the samples was six grams. On opening the chamber of the borer they were rapidly transferred to the nickel-plated brass tubes with screw caps, adopted from the ordinary shaving-soap tube by my colleague, Mr F. Hughes. These tubes have been completely satisfactory. Care was taken to screen the tubes from the sun, both before, after, and during the filling, and they were weighed immediately after the bore had been finished. Drops of perspiration provided another error to avoid.

The drying of the samples was done in the tubes, and standardised as follows. The caps of the tubes having been removed, the latter

<sup>1</sup> To avoid repetition, all measurements downwards from the soil surface are simply stated in centimetres without adding "below soil surface."

were arranged in a circle inside the water-oven at 100° C. A glass pipe descended through the roof of the oven to a level with the tops of the tubes, and equidistant from all of them; this pipe led to the vacuum water-pump, which was kept running continuously during the six hours which drying lasted, and so provided a gentle draught over all the tubes equally, removing the evaporated water.

All determinations are thus fairly comparable, even though the absolute values may not have been obtained. Samples from the loamy layer would dry more rapidly than those from the clayey layer, but the error is not serious, since a sample from any one depth was usually of the same texture at all times. This uniformity is shown on inspection of the deep-soil records for the early summer (Fig. 1).

Fresh sample less dry sample gave water-loss. Dry sample less tare gave dry-weight. The water-content was then expressed as the percentage of water-loss to dry-weight, as in Table I.

All bore-holes were filled up before a watering, to avoid abnormal vertical distribution of water.

#### THE WATER-TABLE.

It will be seen that in the last few bores of the list in Table I the water-content of the soil has risen abnormally, and that traces of an increased water-content appear even before this abnormal rise (Fig. 2*d* and 2*e*). This is due to the rise of the water-table, controlled by infiltration from the Nile itself, about a mile away. In the bore-holes of the 21st and 25th of September, the water had risen on the following days to 107 and 105 cm. respectively. This level is higher than that indicated by the soil analyses, which were plainly not completely saturated until below 120 cm. The discrepancy may be due to capillarity, to a slight artesian effect, or to compression of the soil air between the water-table and the clay above, which would be released by the boring. The effect is not one which concerns us at present, but it should be noted as a discrepancy.

#### SATURATION CONTENT.

Mention of the saturation by the water-table involves reference to the amount of water required to saturate the various layers sampled. In default of precise determinations of this, sufficient indication should be given by the run of the curves themselves. The saturation value is not less than 35 per cent. in the loam.

TABLE I.

Date	Depth in cm.							Number of bore on Plan	Notes
	20	40	60	80	100	120	140		
May 4	24.8	24.2	28.5	32.9	24.9	32.7	30.9	1	
8	22.0	24.2	27.7	32.7	24.3	29.4	30.7	2	
11	3.1	19.3	31.1	28.4	27.0	33.2	29.5	3	
15	8.0	16.6	33.4	27.4	23.3	30.8	30.3	4	
18	5.0	18.3	26.9	30.2	22.3	27.0	31.8	5	
22	6.2	18.3	27.2	32.0	24.9	30.9	31.6	6	
25	10.5	25.1	25.3	32.6	24.4	28.3	34.6	7	
29	6.4	17.4	24.0	30.7	24.4	28.3	27.2	8	
(Irrigated May 30)	26.4	25.6	29.5	29.9	31.3	29.2	24.3	9	
June 2	19.0	23.7	30.1	30.9	27.0	33.3	33.1	10	
5	22.8	23.2	27.8	29.7	29.3	31.8	30.9	11	
8	16.2	20.0	25.7	29.9	28.0	33.4	31.4	12	
16	7.1	10.6	24.8	29.8	23.5	32.2	32.5	13	
19	7.3	13.4	25.4	30.4	21.0	30.7	28.9	14	
23	(17.1?)	14.1?	27.0?	11.5?	17.3	24.8	32.5	15	
(Irrigated June 23)	28.1	26.2	29.7	29.7	28.0	28.9	33.6	16	
26	25.4	25.1	25.9	29.7	25.9	29.3	32.1	17	
29	22.5	22.7	26.6	29.1	28.1	32.0	29.5	18	
July 3	20.4	20.4	26.9	30.9	18.9	30.7	31.1	19	
6	18.0	18.1	20.0	29.4	22.6	24.7	33.1	20	
10	16.7	17.2	13.6	15.7	28.6	28.3	32.0	21	
13	13.1	14.1	13.9	13.3	24.2	29.8	30.9	22	
(Irrigated July 17)	20.9	25.4	26.6	30.8	26.5	32.1	30.3	23	
24	22.2	22.4	19.4	25.1	31.8	28.6	33.1	24	
27	22.8	22.3	18.0	16.8	25.0	30.1	34.2	25	
31	23.0	23.4	23.7	27.4	18.9	32.1	32.1	26	
Aug. 3	17.2	15.6	14.0	12.6	24.6	22.3	30.2	27	
7	17.2	15.1	17.4	20.6	10.8	16.0	30.8	28	
10	—	—	—	—	—	—	—	29	
(Irrigated Aug. 16)	30.7	26.1	23.2	24.8	29.3	29.0	30.9	30	
14	27.3	22.7	20.2	18.9	23.8	25.2	28.9	31	
21	25.2	23.1	—	15.8	10.5	24.6	30.8	32	
24	24.5	22.5	26.3	29.9	24.6	28.4	30.3	33	
28	21.0	15.8	14.6	18.9	32.2	28.4	32.5	34	
31	22.6	16.1	17.6	24.8	30.3	31.4	31.6	35	
Sept. 4	19.2	21.0	26.2	30.2	23.3	32.3	30.1	36	
7	23.6	24.3	26.9	31.2	28.3	28.5	33.0	37	
11	19.7	22.9	24.2	29.8	27.3	30.9	34.8	38	
14	27.7	26.6	25.6	31.4	34.8	34.1	34.2	39	
18	26.8	28.0	28.0	31.2	30.0	31.6	34.3	40	
21	27.1	26.1	28.3	28.1	35.4	29.2	35.5	41	

\* Hard clay, not sand.

1, 2, 5, 6 Clay; 3, 4, 7, 8 Loam.

Surface layers very hard.

Algae on surface.

1-4 Clay; 5 S. Loam; 6 C. Loam; 7, 8 Loam.  
5-8 Sandy.

1, 2, 5 Clay; 3-4 S. Clay; 6, 7 Clay Loam; 8 Loam.

Borer broke at 50 cm.

Twenty hours after irrigation.

\* Sample split in drying.

Note.—The figures show the percentage of water determined in each sample at the depth and on the date indicated. Sampling continued once a week only till November.

*Distribution of water in the soil of a cotton-field.*

May—October, 1912. Giza Botanical Laboratory.

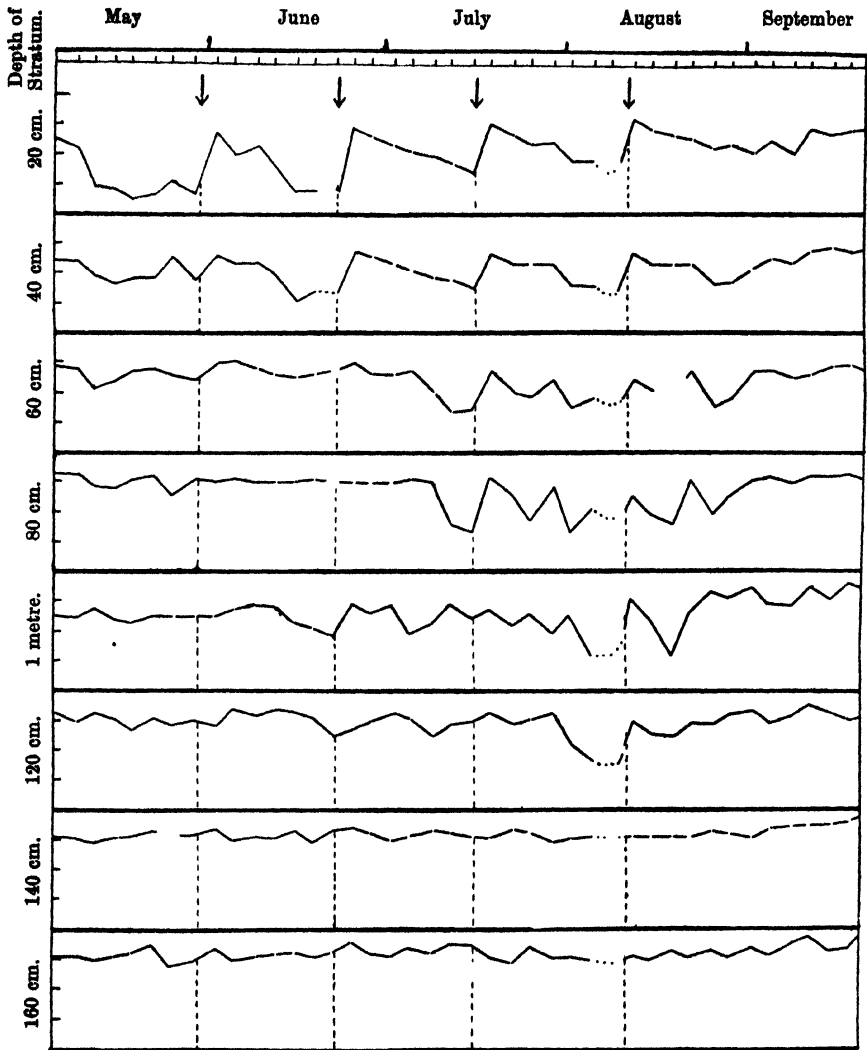


Fig. 1.

*Ordinates* represent percentage of water. Guide-scales on left showing 10%, 20% and 30%.  
*Arrows* denote dates of irrigation.

Plotted from Table I directly.

## THE DRYING EFFECT OF THE ROOT.

On plotting the curves of water-content from the table already given we obtain Fig. 1.

The most striking feature of this figure is the progressive drying of the deeper and deeper layers as the root reaches further and further down, causing a reversal of the Humidity Gradient (Fig. 2*a* and 2*e*). The writer has discussed the general bearing of this on root-function elsewhere<sup>1</sup>, though with much more slender evidence than these curves provide.

The proximity of the water-table to the surface throughout the season is a distinct disadvantage of these records. From May till mid-August it remained fairly steady at a depth of two metres, *i.e.* within 30—40 cm. of the lowest sample taken, or sometimes nearer, *e.g.* July 13th and 17th. The wetting of the lower layers of soil due to this proximity (Fig. 2*d*) serves to obliterate the root-drying effect, which had formerly been recorded at greater depths<sup>1</sup>. On the other hand, we have indirect evidence that the plant draws on the water-table, or rather on the water absorbed by capillarity into the soil immediately above the water-table, for a portion of its water supply.

The same conclusion follows when we consider that the water-table falls during the summer in sites where under-drainage can scarcely take place. In other words, more water may be evaporated from the plant and soil together than is applied in irrigation.

A computation of the total loss of water from the crop during the period covered by the curves, based on the changes in water-content, leads to the same result.

## TOTAL TRANSPIRATION OF THE CROP.

The following table shows the method by which an attempt to estimate this figure has been made.

<sup>1</sup> *Loc. cit.*

TABLE II. *Distribution of Water in Soil.*

Taking means of three borings to eliminate the known soil-diversity at 100 and 120 cm.

A. The week immediately after irrigation.

B. The week immediately before irrigation.

Period	Dates	Depth in cm.							
		20	40	60	80	100	120	140	160
a	A. May 4-11.....	18.6	22.6	25.8	31.3	31.4	31.4	30.4	30.4
	B. „ 22-29.....	7.7	20.3	25.5	29.4	26.9	29.3	29.4	30.2
	Loss .....	10.9	2.3	0.3	1.9	4.5	2.1	1.0	0.2
b	A. June 2-8 .....	22.7	24.2	29.1	30.2	26.9	31.0	31.1	31.2
	B. „ 16-22.....	7.2	12.0	25.1	30.1	20.3?	29.2?	31.3?	31.2?
	Loss .....	15.5	12.2	4.0	0.1	6.6?	1.8?	0.0?	0.0?
c	A. June 26 .....	25.3	24.6	27.1	29.3	27.8	29.1	31.7	32.7
	B. July 10-17.....	16.3	16.5	15.5	19.5	25.1	27.6	32.0	33.6
	Loss .....	9.0	8.1	11.6	9.8	2.2	1.5	(+0.3)	(+0.9)
d	A. July 20-27.....	25.9	23.4	21.3	24.2	27.8	30.3	31.8	30.4
	B. Aug. 3-7 .....	17.2	15.3	15.7	17.6	17.7	19.2	30.5	29.9
	(Two bores only.) Figures too high.) Loss...	8.7	8.1	5.6	6.6	10.1	11.1	1.3	0.5
e	A. Aug. 14-21 .....	27.7	23.6	31.7	19.8	21.2	26.3	30.6	30.7
	B. Aug. 31-Sept. 7...	21.8	20.5	23.6	32.1	27.3	30.7	31.6	31.5
	Loss .....	5.9	3.1	8.1	(+12.3)	(+6.1)	(+4.4)	(+1.0)	(+0.8)

NOTE.—Figures represent percentage of water as in Table I, but each averaged from three consecutive dates as there given.

The means of the three borings made immediately after irrigation have been taken together, in order to eliminate slight soil diversities; then, missing one boring, the means of the three preceding the next irrigation have been taken. The difference gives the loss of water from each layer during the number of days elapsing from group-centre to group-centre. This is then assumed to be a fair mean for the whole period between one irrigation and the next; in all probability the assumption is too low, since the stomata immediately after irrigation remain open longer each day, and so transpire more water, though this again may be partly compensated by the higher humidity of the air.

In any case the figure which we thus obtain for the mean water-loss from soil and plant between one irrigation and the next, is not likely to be too high.

In the case of the five periods here computed, this figure, expressed as a mean of the eight samples, and for an average day, worked out at 0.170, 0.359, 0.366, 0.541 and (+ 0.052) per cent. of the dry-weight of the soil. With the Sp. Gr. of the soil assumed to be 1.0, these figures correspond to 11, 24, 25, 36 and (+ 4) *tons of water per acre per day*.

It remains to allocate this loss to soil-evaporation and to plant-transpiration respectively. For this there is no direct method, but fairly safe indirect conclusions may be drawn from the following considerations. At the beginning of this series of observations the plants are only a few centimetres in height, and most of the surface soil is freely exposed to the sun; the writer has published thermo-electric records<sup>1</sup> of the soil temperature near the surface under such conditions, at an earlier period of the season, showing how stringent the conditions are, and since the mean depth of the root-system at this time is only about 50—60 cm., it follows that a great part of the surface evaporation must be simply from the soil. Within the first two months—May and June—there is a very rapid growth of the stem, and a steady increase in root depth, so that by June 29th the root reaches a depth of about one-and-a-half metres, while the stem is over a metre in height. On this date it was noted that the surface soil of the plot was coated with Cyanophyceae (blue-green algae) which only flourish on surfaces where the evaporation is very low, or is continually compensated by fresh water supply. It would appear that the evaporation from the surface soil after July 1st was almost negligible, and it might be added that routine records taken with the hygrograph in this plot, at 10 cm. from the ground surface, showed that the mean humidity rose very rapidly during June and July, this implying a diminished evaporation, even without the reduction in wind velocity which follows from the interlacing of the plants.

Returning to the conditions during May, we see from the curves (Fig. 2) that even under these, the most stringent conditions, the loss of water is trivial below 40 cm. Making the maximum allowance for surface evaporation from the soil, in the light of these data it would

<sup>1</sup> *Loc. cit.*

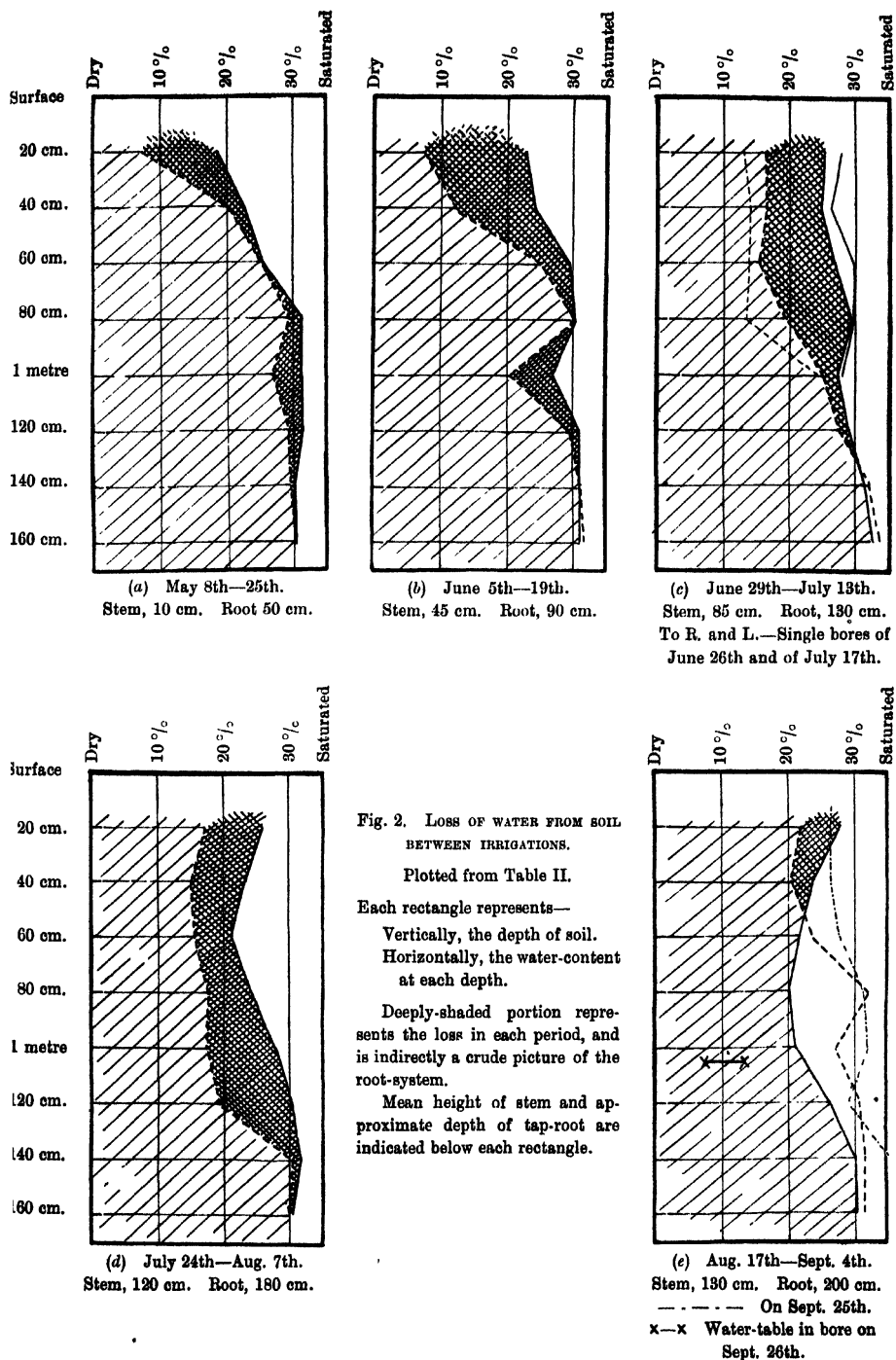


Fig. 2. LOSS OF WATER FROM SOIL  
BETWEEN IRRIGATIONS.

Plotted from Table II.

Each rectangle represents—

Vertically, the depth of soil.

Horizontally, the water-content  
at each depth.

Deeply-shaded portion represents the loss in each period, and is indirectly a crude picture of the root-system.

Mean height of stem and approximate depth of tap-root are indicated below each rectangle.



seem more than fair to estimate that the correction should be somewhat as follows:

TABLE III.

Period	Total loss of water in tons per acre per day	Loss due to	
		Soil evaporation	Transpiration
May 8 } to May 25 }	11	7	4
June 5 } to June 19 }	24	10	14
June 29 } to July 13 }	25	4	21
July 24 } to Aug. 7 }	36	2	34
Aug. 17 } to Sept. 4 }	(+4)	?	

Mean transpiration=18 tons of water per acre per day.

The figures, approximate though they may be, require some comment, in that M. Audebeau, who conducted the only extant series of experiments upon the transpiration of cotton, by weighing plants growing in large tanks, arrived at a mean figure for the same period of 21.9 tons per acre per day<sup>1</sup>. I have elsewhere pointed out<sup>2</sup> several serious objections attaching to this figure, notably that the plants were abnormally large and freely exposed to wind; yet, under normal and undisturbed field conditions, we have reached a figure of the same order as M. Audebeau's. It is one of the most curious examples of compensation of error with which I have yet met, even when allowance is made for the higher evaporimeter reading at Giza as against Korashia. Probably a detailed consideration of stomatal behaviour, of the wind velocity needed to accelerate transpiration, and so forth, would explain the convergence, but it is of more importance to notice that M. Audebeau's figure, now confirmed by the writer, was so high

<sup>1</sup> *State Domains Report, Cairo, 1910.*

<sup>2</sup> *Loc. cit.*

that it necessitated more water for the cotton crop alone than was available in the Nile during the summer. We can only conclude that the deep-rooted plant draws on the water-table, when within reach.

We have now reached this last conclusion by three different lines, and it seems to be quite sound. In one way it is quite to be expected, but it does not seem to have been taken into account before, presumably because the "deep draught" of the plant had not been realised. The drying effect which is shown in this series at 120 cm. might easily, with a deeper water-table, have been followed down to two-and-a-half metres.

It does not follow that the water-table, though utilisable, is of any advantage to the crop above. The existence of a water-table within 3 metres of the surface still implies a limitation of the root-system, and a risk of submergence, with all their attendant evils.

#### GRADIENT OF HUMIDITY.

With the previous discussion of the "surface climate" conditions in mind, the reversal of humidity gradient shown in Fig. 2 becomes easily comprehensible.

The metaphor which the writer formerly<sup>1</sup> devised, of a "functional centre of gravity" in the root-system, becomes very convincing. It had not been suspected previously—to the best of the writer's belief—that the soil would actually become drier as the borer passed deeper into it, down to at least a metre depth.

The abnormal reversal of the gradient between August 14th and September 7th is also worthy of note. It is due solely to the rise of the water-table, but it is shown far above the water-table, and many days before the saturation level actually reached the samples. We might assume that this upward movement of the water was due to capillarity, but it is quite debatable, and indeed highly probable, that the movement is rather a case of direct "thrust" of the rising water upon the overlying water-films, producing a general redistribution of hydraulic pressure.

Had it not been for the patches of clay on the site examined, it might be possible to make some physical calculations from the data at hand.

<sup>1</sup> *Loc. cit.*

## EFFECT OF IRRIGATION.

Consecutive with the last paragraph it may be well to comment on the effect of applying surface water, by irrigation, as shown in these records.

I am informed by local chemists and physicists that some divergence of opinion exists as to the depth at which the effect of surface irrigation is noticeable in the soil. The general conclusion appears to be that the effect is only felt over a limited distance; this is the exact contrary of my own deduction from these records (Fig. 2), and is, to say the least, extremely improbable. The writer is not sufficiently qualified to discuss the matter physically, but it might be pointed out that the introduction of a new component—irrigation water—at the upper end of a system which is in equilibrium between surface-tension, transpiration and gravity, can hardly fail to produce a disturbance throughout the system. Any contrary conclusion must be due to some local abnormality, or to methods which are not sufficiently minute to detect the disturbance.

Inspection of the curves in Fig. 2 shows at once that the effect of each irrigation is felt to the lowest level at which the soil has dried, and that the level at which its effect disappears is that at which no change has been taking place in the soil water-content. Had the water-table been deeper, the effect would undoubtedly have been traceable to a greater depth. But it should be noted that this effect is often of short duration, owing to the rapid desiccation of the soil brought about by the roots, and unless the observations are, as in this case, made at frequent intervals, is likely to escape detection altogether.

## THE PROBABLE ERROR OF SOIL-WATER DETERMINATIONS.

In connection with the previous heading it is of interest to note that very serious errors may arise in the determination of the water condition (and indirectly, of the salt-content) of fields of cotton in Egypt by any method of sampling, if the sampling is intermittent.

The present data are scarcely full enough to enable a statistical statement to be made, but the following result will illustrate the writer's meaning.

The water-content of the samples at 40 and at 60 cm. taken at 13 and at 17 days after watering, may be considered as equivalent

to a series of samples taken half a metre down, over a large field at fifteen days after irrigation, for the purpose of ascertaining its water condition, since irregularities of soil surface and sub-soil structure, which are practically absent in the small plot observed by the writer, would then be involved. If no allowance is made for the seasonal change, shown in this present paper, the P.E. of these samples works out to 3.1 per cent. of actual soil-water.

Similarly, the samples from 100 and 120 cm. give a P.E. of 3.5 per cent. of actual soil-water.

The extreme variation in soil water-content, excluding the water-table, ranges in these experiments from 33 to 10 per cent. At the usual odds of 30:1 for certainty ( $3.5 \times 3.2 = 11\frac{1}{2}\%$ ), the samples at one metre depth may range from one extreme to the other without being significant.

It is highly improbable that any conclusions would ever be drawn from single samples taken blindly in this way, but it is well to bear in mind that the seasonal change should be the first consideration, the post-irrigation change the second, and that totally false estimates might be formed by such random sampling.

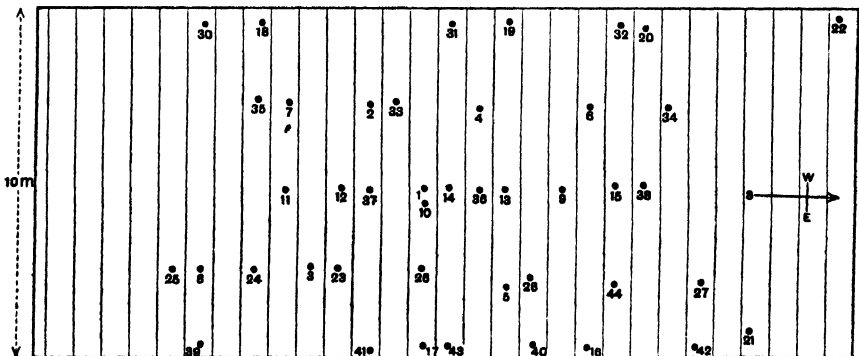


Fig. 3. Plan of Bores. Lines E. and W. represent ridges.

### CONCLUSION.

This paper describes and discusses a series of soil-water determinations made on an area of  $20 \times 10$  metres in a field of cotton at Giza, in Egypt.

The especial feature of the determinations was their frequency. They were made every three and four days alternately, and at twenty centimetre intervals down to 160 cm.

Fairly smooth statistical results were thus obtained.

The chief conclusions drawn are as follows :

A. The depth of the root may be roughly traced by its drying effect on the soil.

B. This drying, combined with a change in the surface climate, causes a reversal of the Humidity Gradient, so that deep soil is drier than surface soil in September.

C. The rate of evaporation from this field of cotton plants averaged about 20 tons of water a day, from May to October.

The results of M. Audebeau in this respect are substantially correct, though circumstantially in error.

D. Application of irrigation water to the surface is felt to an indefinite depth. Absence of evidence to this effect is due to imperfection of method.

E. Determination of soil water-content in an Egyptian cotton field by random sampling is almost worthless, unless due regard is paid to the seasonal variation.

F. The water of the water-table, when within two metres of the surface, may be utilised by the crop, but this conclusion does not vitiate any previous conclusions drawn by the writer as to the prejudicial effects of saturated soil.

G. A rise of the water-table is analogous to surface irrigation, and there is some indication of a direct hydraulic thrust in both cases.

The observations and operations were entirely conducted personally until July 18th, when they were taken over by Mr F. S. Holton during my absence on leave. I am very much indebted to Mr Holton for contriving to carry on the extra work in a very busy summer.

# THE EFFECT OF BASTARD TRENCHING ON THE SOIL AND ON PLANT GROWTH.

By SPENCER UMFREVILLE PICKERING, M.A., F.R.S. AND  
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BASTARD Trenching as ordinarily performed consists of two distinct operations; loosening the lower spit of soil, and digging into it farmyard manure or other fertilising material. A considerable volume of data has been accumulated to show the effect of the addition of farmyard manure to soil, but little is known of the effect of loosening the bottom spit, either on the soil or on the plant.

The experiments described in this paper were made on plots that had been bastard trenched to a depth of three spits, but not manured. The first and second spits were put back in their natural order, but no manure was added. The experiment, therefore, deals simply with deep cultivation effect, and is not complicated by any disturbing factors due to the operation of the manure.

Four distinct types of soil were investigated; a light sand, two loams (both rather heavy) and a strong clay. The bulk of the experiments extended over the four seasons from March 1909 to the end of 1912, a period which included the unusually hot dry summer of 1911, the cold wet summers of 1909 and 1912, and the season 1910 which was of intermediate character.

Samples of soil were periodically taken for determinations of moisture and nitrate, and observations were made on the growth of fruit trees in the plots.

The results show that trenching has very little effect on the moisture content of the soils. There is some indication that it facilitates percolation from the surface to the subsoil on heavy loams and clay, but the indication is not very marked, and only comes out with certainty

on looking over the whole of the results. It also somewhat increases the subsoil moisture. No other tendency could be distinguished, and we must regard trenching as a very minor factor in determining the water supply to the plant.

Nor did it appear to lead to any marked increases in the store of nitrate in the soil. There were small gains on the sand and rather larger gains on the clay, which persisted over all the four seasons, but these were never very great, and not much above the error of the experiment. Trenching did not appear to alter the distribution of the nitrate between the surface and the subsoil.

The behaviour of the plant furnishes a sensitive index to the changes in the soil. Here, however, the indications are not much more definite than those given by the determinations of water and nitrate. An increased leaf-size (generally of small dimensions) is shown in three out of the four cases in which this feature was measured, but in the fourth there is a reduction; an increased growth was recorded in three cases, but a reduction in two cases; whilst in the two instances where there were records of crops, both are in favour of leaving the ground untrenched.

Putting together the whole of the evidence, the effect of bastard trenching on the soil *when unaccompanied by manuring* is seen to be only small. Beyond a tendency to facilitate the drainage of water from the top spit to the lower spit in the clays and the heavy loams, and slightly to increase the nitrates, no definite change seemed to be produced. The effect on the growth of trees appeared to depend largely on the character of the seasons following the trenching and planting, as was exemplified by the different results obtained in the same plot of ground after trenching in 1895, and after retrenching in 1910. The practical conclusion may be drawn that bastard trenching by itself, done without addition of manure to the bottom spit, is not likely to bring about any sufficient change in the soil to justify the trouble and expense of the operation. Of course if there is a pan to be broken the case is different; but, where there is no pan, the main use of bastard trenching seems to be that it affords an opportunity for adding manure or other fertilising material to the bottom spit. Unless advantage is taken of this, the real benefit of the process is missed.

## EXPERIMENTAL.

In all the present experiments the first and second spits of soil were removed, the third spit was broken up, but not removed, and then the second and first spits were replaced in their natural order. Nothing was buried in the trench.

The trenched and untrenched plots were contiguous to each other, and were similarly treated as to cultivation and manuring, a general artificial manure being applied in all cases, except in the experiment at Rothamsted.

*The Soils.*

(a) *The Sand.* This was situated at Millbrook, Bedfordshire, on the greensand formation, where the soil is very poor and sandy. The upper and lower spits differ very little in mechanical composition or in texture. There were three trenched plots here, with the corresponding untrenched plots adjoining; one (first series) was trenched in February 1909, another (second series) in March 1910, and the third in March 1912. In the first two cases the plots measured 100 square feet, the third was 484 square feet. The second plot was added to ascertain whether the results obtained on the first plot had been affected by the lie of the ground; it was therefore arranged so that the two trenched and the two untrenched plots should lie diagonally towards one another, as indicated in the following diagram:

Trenched	Untrenched
Untrenched	Trenched

The mechanical analysis of the soils is given in Table I.

(b) *The Loams.* Two sets of experiments were carried out, one at the Woburn Experimental Fruit Farm, Ridgmont, the other at Rothamsted. The Ridgmont soil is a heavy loam situated on the Oxford clay. It was trenched in 1894 and retrenched in 1910. Each plot measured 250 ft. by 12 ft. The Rothamsted plots were situated



on a part of Broadbalk field where lucerne, preceded by potatoes and wheat, had been grown. The central plot, measuring 80 ft. by 20 ft., was trenched in February 1909, the untrenched plot, of the same total area, was subdivided so as to lie on either side of the trenched section. This soil is also heavy, and of the clay-with-flints formation overlying the chalk.

TABLE I. *Mechanical Analysis of Soils.*

	Sandy Soil (Millbrook)	Loam (Ridgmont)	Loam (Rothamsted)	Clay (Ridgmont)
Surface				
	%	%	%	%
Fine Gravel .....	8.2	1.0	1.3	0.4
Coarse Sand .....	68.2	54.8	9.5	8.6
Fine Sand .....	9.2	11.5	22.7	3.8
Silt .....	5.6	11.8	32.3	9.1
Fine Silt .....	4.9	6.7	8.9	11.1
Clay .....	5.0	9.3	12.9	43.0
Loss on Ignition	4.2	5.3	7.7	18.6
Subsoil				
Fine Gravel .....	2.2	1.6	2.6	0.2
Coarse Sand .....	70.8	53.0	8.3	16.8
Fine Sand .....	8.8	11.5	24.5	10.7
Silt .....	4.9	10.9	27.5	10.8
Fine Silt .....	5.0	4.7	10.6	10.0
Clay .....	5.2	10.9	15.0	32.0
Loss on Ignition	4.2	5.2	7.1	13.8

(c) *The Clay.* This is a heavy clay soil, on the Oxford clay formation at Ridgmont, and shows the unusual phenomenon of containing more fine material in the surface than in the subsoil. As it is very heavy and low-lying, it was drawn out into ridges and furrows, the former, on which the trees were planted, being 7 ft. 6 in. apart. This experiment really deals with the effect of retrenching, because the ground had all been trenched in 1894; the portion which was re-trenched in November 1905 lay on the side nearer to a brook.

### *The Trees.*

(a) *In the sandy soil.* In the three trenched plots on the light Millbrook soil, dwarf trees, of five different varieties, two years of age, were planted in 1912: five in each of the earlier trenched plots, and 40 in the last trenched one. A like number of trees were planted in

the corresponding untrenched plots. Details as to the behaviour of these trees in the following season are entered in Table V, the results with the different varieties being given separately in the case of the larger plots only.

(b) *In the loams.* The whole of the ground at the Fruit Farm, Ridgmont, except one plot measuring 250 by 50 feet, was trenched in the summer of 1894. In the untrenched plot 18 dwarf apple trees of three different varieties—Bramley, Cox and Potts—were planted in the following winter, and the general behaviour of similar trees in four other plots in the adjoining trenched ground, as compared with that of the trees in the untrenched plot, is illustrated by the data in Table V (Ridgmont, first series). In 1910, after these trees had been removed, 40 two-year-old trees of Gascoyne's Scarlet Seedling were planted in the portion which had never been trenched, and 40 in the adjoining plots trenched in 1894, but one half of these plots were retrenched in 1910, immediately before the planting. This constituted the second series. The trees in this case were rammed on planting, but they were not so in any of the other experiments. Though this ramming might naturally be expected to have diminished any effect due to the trenching, it will be seen that this, apparently, has not been the case, these trenched plots showing a greater effect due to the trenching than in any other instance.

At Rothamsted the plots were planted in February 1909 with eight standard and 30 dwarf apple trees, six dwarf pears and six dwarf plum trees, 20 gooseberry and 20 currant bushes. No manure was supplied.

(c) *In the clays.* Four standard and five dwarf apple trees, four standard and five dwarf plum trees, and five dwarf pear trees were planted in December 1905.

*The Moisture Determinations.* Samples were taken in the field by the cylindrical borer and allowed to dry in a hot chamber at 37°—40° C. The mean results for the various soils over the whole period are given in Table II, and the fuller results in Table III. In the latter table no significance is attached to differences less than  $\pm 0.3$  per cent. in the case of the sands and loams, or  $\pm 0.6$  per cent. in the case of the clay, experience having shown that variations of this order come within the limits of experimental error. The small effect of trenching on the moisture content is evident. The trenched and untrenched plots on the sand are practically equally moist, when the results of the two sets of plots are considered. In Series I the trenched plot has

a drier subsoil than the untrenched, but not in Series II. On the loam the trenched surface soils tend to be distinctly moister in dry weather (*e.g.* August 1911 and May 1912) and are on some occasions rather drier in wet weather (*e.g.* September and November 1909, January 1912); the trenched subsoil in Series II is, on the other hand, somewhat wetter in wet weather (indicating increased percolation) and drier in dry weather than the untrenched. At Rothamsted the trenched soil is throughout the moister. On the clay soil the consistent behaviour is seen in the subsoil, which is always wetter on the trenched plots than on the untrenched. This result may be partly due to the relative positions of the plots, the trenched plot, as already stated, lying towards the stream. But going over the whole of the figures there is an unmistakable though small tendency for trenching to increase the subsoil moisture. The surface soil is sometimes wetter on the trenched plot, but in dry weather it is often markedly drier.

TABLE II. *Mean Percentages of Moisture in the Trenched and Untrenched Soils.*

	1909		1910		1911		1912		Mean	
	Trenched	Untrenched	Trenched	Untrenched	Trenched	Untrenched	Trenched	Untrenched	Trenched	Untrenched
<i>Sandy Soil</i>										
Millbrook, 1st Series. Top 9"	11.9	11.9	8.4	8.4	4.8	4.1	6.7	6.0	7.9	7.5
" " 9"—18"	8.8	9.9	7.4	7.5	4.5	4.6	5.9	7.6	6.5	7.4
" 2nd Series. Top 9"	—	—	9.0	8.8	4.6	4.8	6.1	6.8	6.1	6.4
" " 9"—18"	—	—	6.6	6.2	4.2	4.1	5.6	5.2	5.2	5.0
<i>Loam</i>										
Ridgmont, 1st Series. Top 9"	14.0	14.1	12.2	11.6	7.5	7.2	9.6	9.7	10.5	10.5
" " 9"—18"	13.3	13.2	11.7	11.2	8.0	7.9	9.5	10.1	10.9	10.5
" 2nd Series. Top 9"	14.2	14.2	11.6	11.2	8.7	8.1	10.2	9.2	10.8	10.2
" " 9"—18"	14.7	14.3	13.1	12.6	9.7	10.5	11.8	10.7	11.8	11.7
Rothamsted. Top 9"	15.3	15.1	14.9	14.2	12.8	12.1	13.7	13.6	14.1	13.5
" 9"—18"	16.2	15.3	15.4	14.6	13.9	12.8	15.1	14.0	15.1	13.9
<i>Clay</i>										
Ridgmont Ridges. Top 13"	28.2	27.7	23.4	23.6	16.8	18.7	18.2	19.6	21.4	22.4
" " 13"—22"	32.1	29.2	28.4	27.5	23.8	20.5	23.1	22.2	26.6	24.8
" Furrows. Top 5"	28.0	27.1	21.9	23.6	19.3	20.3	21.1	20.3	22.7	22.7
" " 5"—14"	33.1	31.4	30.6	28.9	25.2	22.6	25.0	23.8	28.2	26.3

TABLE III. Water present at different dates in the Trenched and Untrenched Soils.  
Percentage of Water.

		1909										1910		
Description		Date of Trenching	March 13th	May 6th	July 14th	Sept. 1st	Sept. 17th	Nov. 17th	June 14th	Aug. 2nd	Sept. 11th			
<i>Sandy Soil</i>														
Millbrook.	1st Series. Top 9" Untrenched ..	Feb. 1909	14.0	9.4	12.1	9.4	12.4	14.2	8.4	9.7	7.2			
"	" " Trenched ..		14.0	10.1	11.4	9.1	12.8	14.2	8.7	9.0	7.6			
"	" " 9"—18" Untrenched ..		10.5	9.1	11.6	8.8	7.6	11.9	7.4	8.4	6.8			
"	" " Trenched ..		10.0	8.0	9.6	6.8	7.8	10.4	7.5	7.9	6.9			
"	2nd Series. Top 9" Untrenched ..	Mar. 1910							9.0	8.9	8.4			
"	" " Trenched ..								9.1	9.4	8.6			
"	" " 9"—18" Untrenched ..								6.3	6.6	5.8			
"	" " Trenched ..								6.1	7.1	6.5			
<i>Loam</i>														
Ridgmont.	1st Series. Top 9" Untrenched ..		14.6	11.2	13.7	14.0	15.0	16.2	11.6	11.9	11.4			
"	" " Trenched ..	1894	14.4	—	13.4	13.4	14.2	14.7	11.8	12.7	12.1			
"	" " 9"—18" Untrenched ..		13.9	12.8	13.0	12.6	12.9	14.0	12.0	11.3	10.3			
"	" " Trenched ..		13.9	—	13.6	12.2	13.0	13.7	11.2	12.1	11.7			
"	2nd Series. Top 9" Untrenched ..		—	—	13.2	13.0	14.4	16.3	11.0	11.4	11.3			
"	" " Trenched ..	Retrenched	—	—	12.9	13.0	14.7	16.0	11.6	11.8	11.6			
"	" " 9"—18" Untrenched ..	1910	—	—	12.6	15.8	13.2	15.5	11.8	13.2	12.9			
"	" " Trenched ..		—	—	12.8	16.5	14.3	15.1	13.1	13.1	13.1			
<i>Clay</i>														
Rothamsted.	Top 9" Untrenched ..		April 6th	May 7th	July 6th	Oct. 28th			May 30th	July 27th	Sept. 20th			
"	" " Trenched ..	1909	15.0	14.3	13.7	17.7	—	—	13.5	14.3	14.9			
"	" " 9"—18" Untrenched ..		15.5	13.7	14.3	17.9	—	—	13.6	15.1	15.1			
"	" " Trenched ..		15.6	16.0	15.0	18.2	—	—	14.7	13.9	15.2			
"	" " Retrenched ..								15.0	15.4	15.7			
<i>Clay</i>														
Ridgmont.	Ridges. Top 13" Trenched ..	1894	27.3	25.7	27.5	27.6	26.5	31.9	34.1	22.7	24.0			
"	" " Retrenched ..	1905	29.5	24.7	27.7	28.4	26.9	31.8	19.7	24.6	25.8			
"	" " 13"—22" Trenched ..	1894	30.7	26.7	28.3	29.1	27.3	33.3	27.2	27.5	27.7			
"	" " Retrenched ..	1905	33.7	31.6	30.3	31.4	31.3	34.1	28.3	29.3	27.7			

TABLE III—(continued).

		1912											
		1911											
Description	Date of Trenching	May 4th	May 22nd	June 15th	July 13th	Aug. 9th	Sept. 7th	Jan. 13th	April 1st	May 3rd	June 26th	July 19th	Sept. 27th
<i>Sandy Soil</i>													
Millbrook. 1st Series. Top 9" Untrenched	Feb. 1909	8.4	7.2	2.8	1.5	1.3	3.3	9.5	9.4	4.4	5.6	2.2	4.9
" " " Trenched		9.2	6.8	3.0	4.1	1.2	4.3	10.2	9.4	6.8	5.9	3.1	4.9
" " " 9"—18" Untrenched		6.9	7.5	4.1	3.1	1.9	4.4	8.9	9.2	(6.6)	6.5	4.9	8.6
" " " Trenched		6.6	7.2	3.9	5.7	3.0	5.0	7.8	7.6	4.2	6.6	6.9	6.2
" 2nd Series. Top 9" Untrenched	Mar. 1910	9.5	8.1	4.2	1.1	2.4	3.8	(8.0)	9.8	8.4	6.6	2.3	6.9
" " " Trenched		9.4	8.2	3.5	1.7	1.3	3.5	9.1	9.2	4.6	5.3	2.5	5.9
" " " 9"—18" Untrenched		6.1	6.1	—	1.7	2.6	4.2	7.0	7.7	4.0	5.3	1.8	5.2
" " " Trenched		6.5	6.9	3.7	1.3	2.0	4.7	(7.9)	8.0	—	6.0	3.1	5.5
<i>Loam</i>													
Ridgmont. 1st Series. Top 9" Untrenched		11.1	9.9	8.7	6.1	3.4	4.4	16.3	10.8	4.2	10.5	6.5	9.7
" " " Trenched	1894	10.6	9.8	8.4	7.3	4.5	4.5	14.6	9.9	6.6	10.0	7.6	8.8
" " " 9"—18" Untrenched		11.4	10.4	9.3	7.3	4.0	5.1	14.6	10.6	6.7	10.8	8.6	9.7
" " " Trenched		11.1	10.5	8.7	8.1	4.8	5.0	14.5	10.7	6.1	9.0	7.2	(10.7)
" 2nd Series. Top 9" Untrenched		11.5	9.6	8.3	7.3	5.8	6.2	15.7	10.7	6.6	9.1	6.2	6.7
" " " Trenched	Retr. 1910	11.2	9.6	9.5	7.6	7.3	7.2	16.8	10.4	8.4	9.7	6.6	9.1
" " " 9"—18" Untrenched		11.7	12.8	11.4	10.5	7.4	9.3	15.1	10.1	9.1	10.3	9.9	9.8
" " " Trenched		11.8	11.9	9.9	8.3	8.3	7.9	16.3	11.0	8.7	11.2	9.6	10.7
Rothamsted. Top 9" Untrenched		May 17th	June 8th	July 27th	Sept. 13th			Feb. 15th	April 30th	May 30th	June 26th	July 22nd	Sept. 25th
" " " Trenched	1909	15.6	12.8	13.0	6.8	—	—	18.2	11.0	10.7	14.0	14.6	13.4
" " " 9"—18" Untrenched		16.1	14.0	13.7	7.6	—	—	17.9	11.1	11.7	14.3	15.3	12.3
" " " Trenched		16.4	14.0	13.2	7.5	—	—	17.5	12.6	12.1	14.4	13.3	14.1
" " " Trenched		16.9	14.4	13.8	10.4	—	—	18.2	14.4	13.3	15.3	14.6	14.7
<i>Clay</i>													
Ridgmont. Ridges. Top 13" Trenched	1894	24.5	20.7	19.8	18.2	13.0	15.7	27.5	20.8	(16.2)	17.5	14.9	17.5
" " " Retrenched	1905	23.3	15.3	18.2	16.6	14.4	12.8	28.5	19.0	15.1	18.4	13.9	14.1
" " " 13"—23" Trenched	1894	23.8	24.5	24.6	16.0	16.6	17.3	29.7	25.4	(20.6)	21.1	17.7	17.2
" " " Retrenched	1905	27.1	26.4	26.7	22.0	20.5	20.0	30.8	26.1	22.4	20.0	19.7	19.8

*The Nitrate Determinations.* A known weight of the dried soil (150—200 grams) was pounded up and extracted with about 800 c.c. of water on a Büchner funnel, the extract was concentrated in presence of a little magnesia, acidified and reduced by the zinc-copper couple. The ammonia was then determined by distillation into standard acid in the usual way. Table IV gives the average results for the different years. No significance attaches to less than one part per million in the case of sands and loams, or two parts in the case of clays. This is for

TABLE IV. *Mean parts per million of Nitrates in the Trenched and Untrenched Soils.*

			1909		1910		1911		1912		Mean	
			Trenched	Untrenched	Trenched	Untrenched	Trenched	Untrenched	Trenched	Untrenched	Trenched	Untrenched
<i>Sandy Soil</i>												
Millbrook.	1st Series.	Top 9"	5	4	4	2	5	4	4	3	4	3
"	"	9"—18"	4	3	3	2	3	4	3	2	3	3
"	2nd Series.	Top 9"	—	—	7	7	4	4	7	5	6	5
"	"	9"—18"	—	—	5	4	3	3	3	3	4	3
<i>Loam</i>												
Ridgmont.	1st Series.	Top 9"	3	6	2	3	9	9	8	4	6	6
"	"	9"—18"	3	3	2	3	5	5	4	3	4	4
"	2nd Series.	Top 9"	5	5	10	11	15	14	4	6	8	9
"	"	9"—18"	4	3	6	4	10	9	3	4	6	5
Rothamsted.	Top 9"		6	6	12	13	15	14	7	7	9	10
"	9"—18"		6	6	7	7	12	13	6	6	8	8
<i>Clay</i>												
Ridgmont.	Ridges.	Top 13"	7	6	8	7	15	11	10	6	11	8
"	"	13"—22"	8	7	7	5	10	7	10	5	9	6
"	Furrows.	Top 5"	5	5	7	6	10	6	6	3	7	5
"	"	5"—14"	4	4	7	3	8	5	5	3	6	4

the individual determinations: for the average results given in the table a difference of one part per million is probably real. The effect of trenching on nitrate production is only clearly marked in the clay soils; here it generally leads to an increase in the stock of nitrate both in the surface and the subsoil. It is difficult to account for the increase in the surface soil. The increase in the subsoil, when it occurs, may be associated with the increased aëration following after trenching; it is less likely to be due to the increased percolation. We could find but

little definite evidence that the small extra percolation on the trenched ground really affected the distribution of the nitrates.

*The Amount of Plant Growth.* The mode of taking the leaf size and the length of new wood formed is fully described in the *Report of the Woburn Experimental Fruit Farm* (1900, p. 114). Briefly, the leaf size is determined by weighing the sixth leaves from the ends of the shoots, while the length of new wood is determined by direct measurement. As the results show considerable variation in passing from tree to tree they are calculated as proportionate percentages, *i.e.* the values for the trenched and untrenched plots are calculated as percentages of the smaller result, whichever it happens to be, and then designated + or - according as the trenched plot gives the higher or the lower result. The increase in weight of the tree is first calculated as a percentage of the original weight and then worked out as percentages of the smaller result.

Owing to the variation in the crops from the individual trees or bushes it becomes necessary for the determination of these means to weight the individual results according to the actual magnitude of the crop. This has been done for the three values given in Table V.

TABLE V. *Effect of Trenching on the growth of Fruit Trees.*

A. *Sandy Soil.* Ground trenched 1909—1912 as stated. Trees planted 1912.

	Percentage difference in favour of trenching		
	Leaf size	Length of new wood	Increase in weight of the trees
Trenched in 1909 ...	+ 11	- 54	- 19
„ 1910 ....	- 4	- 20	+ 41
„ 1912 .....	—	—	—
Gascoyne .....	+ 2	- 43	- 33
Grosvenor .....	- 3	+ 31	- 85
Worcester .....	+ 11	- 43	+ 35
King .....	+ 35	- 67	+ 33
Newton .....	- 14	- 56	+ 13
Mean .....	+ 4	- 37	+ 5

B. *The Loams*. I. Ridgmont. 1st Series, 1894—1904 and 1908. Ground trenched during the summer of 1894. Trees planted winter 1894.

Feature examined	Percentage difference in favour of trenching
Leaf size : 10 years 1895—1904 .....	+ 10
Size of the trees in 1899 .....	+ 4
"    "    1904 .....	+ 4
"    "    1908 .....	+ 2
Weight of the trees after 11 or 15 years ..	+ 3
"    "    during first 10 years...	- 21

Average of height, spread of branches and girth of stem.

Results with Bramley and Cox alone available.

2nd Series. Same ground but part was retrenched in 1910 and replanted immediately afterwards.

	Percentage difference in favour of trenching						
	Leaf size			Length of new wood			
	1911	1912	Mean	1910	1911	1912	Mean
Trenched in 1894	- 16	- 11	- 14	+ 28	+ 15	+ 25	+ 23
Retrenched in 1910	- 3	+ 3	0	+ 100	+ 61	+ 56	+ 72

II. Rothamsted. Ground trenched Feb. 1909. Trees planted directly afterwards.

Trees	Percentage difference in favour of trenching									
	Length of new wood					Weight of crops				
	1909	1910	1911	1912	Aver.	1909	1910	1911	1912	Aver.
Standard Apples A. (4)	+ 48	- 37	- 14	- 18	- 5	—	+ 273	+ 108	- 3	+ 126
"    "    B (4)	+ 7	+ 36	+ 1	+ 1	+ 11	—	+ 91	—	+ 77	+ 84
Dwarf Apples A. (10) ...	+ 48	+ 21	+ 5	- 4	+ 17	—	- 7	+ 80	- 36	+ 12
"    "    B. (10) ...	+ 7	- 34	- 20	- 40	- 22	—	- 24	+ 27	- 32	- 9
"    "    C. (10) ...	+ 24	+ 36	- 59	- 16	- 4	—	+ 19	+ 74	- 30	+ 21
Dwarf Pears (6).....	- 12	- 9	- 4	- 28	- 13	—	—	—	—	—
"    Plums (6) .....	+ 11	+ 29	+ 35	+ 85	+ 40	—	—	—	—	—
Gooseberries A. (10).....	+ 36	- 166	- 148	- 6	- 71	+ 31	+ 12	+ 33	+ 30	+ 26
"    "    B. (10).....	- 113	- 122	- 52	+ 27	- 65	- 6	- 6	- 29	- 25	- 17
Black Currants A. (10)...	- 64	- 187	- 128	- 189	- 142	{ + 51 }	—	- 103	- 23	- 25
"    "    B. (10)...	- 9	- 78	- 56	- 118	- 65		—	- 79	- 78	- 35
Mean of large fruit .....	+ 18	+ 6	- 8	- 3	+ 3	—	—	+ 40	- 28	+ 4
"    small " .....	- 50	- 136	- 96	- 72	- 88	+ 14	- 1	- 38	- 22	- 12
General mean .....	- 5	- 46	- 40	- 28	- 30	—	- 1	+ 1	- 25	- 8





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